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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/52, C12N 5/10, A61K 38/19	A2	(11) International Publication Number: **WO 98/49300** (43) International Publication Date: 5 November 1998 (05.11.98)

(21) International Application Number: PCT/US98/07801

(22) International Filing Date: 20 April 1998 (20.04.98)

(30) Priority Data:
08/842,984 25 April 1997 (25.04.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: TRUNCATED VEGF-RELATED PROTEINS

VEGF-B

F/L	PVSQFDGPSHQKKVVPWIDVYTRAT
(1)	PSHQKKVVPWIDVYTRAT
(2)	KVVPWIDVYTRAT
(3)	PWIDVYTRAT
(4)	IDVYTRAT
(5)	YTRAT
(6)	RAT
F/L	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(1)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(2)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(3)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(4)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(5)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(6)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
F/L	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(1)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(2)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(3)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(4)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(5)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(6)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK

(57) Abstract

The present invention provides novel truncated forms of vascular endothelial growth factor-related proteins (VRPs or VRPs) which are useful for the stimulation of angiogenesis *in vitro* and *in vivo*. The invention also provides nucleic acids encoding such novel truncated VRPs and methods of producing truncated VRPs. Pharmaceutical compositions comprising truncated VRPs and methods of gene therapy using the nucleic acids which code for truncated VRPs may be useful for the treatment of heart disease and for wound healing.

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DESCRIPTION

TRUNCATED VEGF-RELATED PROTEINS

Field Of The Invention

The present invention relates to novel truncated forms of vascular endothelial growth factor (VEGF)-related proteins. More particularly, the invention relates to N-terminally truncated VEGF-related proteins that are substantially free of other proteins. Such truncated VEGF-related proteins may be used to stimulate angiogenesis in vivo and in vitro.

The invention also relates to nucleic acids encoding such novel truncated VEGF-related proteins, cells, tissues and animals containing such nucleic acids; methods of treatment using such nucleic acids; and methods relating to all of the foregoing.

Background

Vascular endothelial growth factors (VEGFs), also called vascular permeability factors (VPFs), are a family of proteins that are produced by many different cell types in many organs and act in a highly selective manner to stimulate endothelial cells almost exclusively (reviewed in Ferrara et al., Endocr. Rev. 13:18-32, (1992); Dvorak et al., Am. J. Pathol. 146:1029-39, 1995; Thomas, J. Biol. Chem. 271:603-06, 1996). These publications, and all other publications referenced herein, are hereby incorporated by reference in their entirety.

When tested in cell culture, VEGFs are potently mitogenic (Gospodarowicz et al., Proc. Natl. Acad. Sci. USA 86:7311-15, 1989) and chemotactic (Favard et al., Biol. Cell 73:1-6, 1991). Additionally, VEGFs induce plasminogen activator, plasminogen activator inhibitor, and plasminogen activator receptor (Mandriota et al., J. Biol. Chem. 270:9709-16, 1995; Pepper et al., 181: 902-06, 1991), as well as collagenases (Unemori et al., J. Cell. Physiol. 153:557-62, 1992), enzyme systems that regulate invasion of growing capillaries into tissues. VEGFs

also stimulate the formation of tube-like structures by endothelial cells, an in vitro example of angiogenesis (Nicosia et al., Am. J. Pathol., 145:1023-29, 1994).

In vivo, VEGFs induce angiogenesis (Leung et al., Science 246:1306-09, 1989) and increase vascular permeability (Senger et al., Science 219:983-85, 1983). VEGFs are now known as important physiological regulators of capillary blood vessel formation. They are involved in the normal formation of new capillaries during organ growth, including fetal growth (Peters et al., Proc. Natl. Acad. Sci. USA 90:8915-19, 1993), tissue repair (Brown et al., J. Exp. Med. 176:1375-79, 1992), the menstrual cycle, and pregnancy (Jackson et al., Placenta 15:341-53, 1994; Cullinan & Koos, Endocrinology 133:829-37, 1993; Kamat et al., Am. J. Pathol. 146:157-65, 1995). During fetal development, VEGFs appear to play an essential role in the de novo formation of blood vessels from blood islands (Risau & Flamme, Ann. Rev. Cell. Dev. Biol. 11:73-92, 1995), as evidenced by abnormal blood vessel development and lethality in embryos lacking a single VEGF allele (Carmeliet et al., Nature 380:435-38, 1996). Moreover, VEGFs are strongly implicated in the pathological blood vessel growth characteristic of many diseases, including solid tumors (Potgens et al., Biol. Chem. Hoppe-Seyler 376:57-70, 1995), retinopathies (Miller et al., Am. J. Pathol. 145:574-84, 1994; Aiello et al., N. Engl. J. Med. 331:1480-87, 1994; Adamis et al., Am. J. Ophthalmol. 118:445-50, 1994), psoriasis (Detmar et al., J. Exp. Med. 180:1141-46, 1994), and rheumatoid arthritis (Fava et al., J. Exp. Med. 180:341-46, 1994).

VEGF expression is regulated by hormones (Schweiki et al., J. Clin. Invest. 91:2235-43, 1993) growth factors (Thomas, J. Biol. Chem. 271:603-06, 1996), and by hypoxia (Schweiki et al., Nature 359:843-45, 1992, Levy et al., J. Biol. Chem. 271:2746-53, 1996). Upregulation of VEGFs by hypoxic conditions is of particular importance as a compensatory mechanism by which

tissues increase oxygenation through induction of additional capillary vessel formation and resulting increased blood flow. This mechanism is thought to contribute to pathological angiogenesis in tumors and in retinopathies. However, upregulation of VEGF expression after hypoxia is also essential in tissue repair, e.g., in dermal wound healing (Frank et al., J. Biol. Chem. 270:12607-613, 1995), and in coronary ischemia (Banai et al., Cardiovasc. Res. 28:1176-79, 1994; Hashimoto et al., Am. J. Physiol. 267:H1948-H1954, 1994).

The potential of VEGF to pharmacologically induce angiogenesis in animal models of vascular ischemia has been shown in the rabbit chronic limb ischemia model by demonstrating that repeated intramuscular injection or a single intra-arterial bolus of VEGF can augment collateral blood vessel formation as evidenced by blood flow measurement in the ischemic hindlimb (Pu, et al., Circulation 88:208-15, 1993; Bauters et al., Am. J. Physiol. 267:H1263-71, 1994; Takeshita et al., Circulation 90 [part 2], II-228-34, 1994; Bauters et al., J. Vasc. Surg. 21:314-25, 1995; Bauters et al., Circulation 91:2802- 09, 1995; Takeshita et al., J. Clin. Invest. 93:662-70, 1994). In this model, VEGF has also been shown to act synergistically with basic FGF to ameliorate ischemia (Asahara et al., Circulation 92:[suppl 2], II-365-71, 1995). VEGF was also reported to accelerate the repair of balloon-injured rat carotid artery endothelium thereby inhibiting pathological thickening of the underlying smooth muscle layers, and thus maintaining lumen diameter and blood flow (Asahara et al., Circulation 91:2793-2801, 1995). VEGF has also been shown to induce EDRF (Endothelium-Derived Relaxing Factor (nitric oxide))-dependent relaxation in canine coronary arteries, thus potentially contributing to increased blood flow to ischemic areas via a secondary mechanism not related to angiogenesis (Ku et al., Am. J. Physiol. 265:H586-H592, 1993). Together, these data provide compelling evidence

for a potential therapeutic role of VEGFs in wound healing, ischemic diseases and restenosis.

The VEGF family of proteins is comprised of at least 4 members VEGF-121, VEGF-165, VEGF-189, and VEGF-206. The originally characterized VEGF is a 34-45 kDa glycosylated protein which consists of 2 identical subunits of 165 amino acid residues (Tischer et al., Biochem. Biophys. Res. Commun. 165:1198-1206, 1989). The VEGF-165 cDNA encodes a 191-residue amino acid sequence consisting of a 26-residue secretory signal peptide sequence, which is cleaved upon secretion of the protein from cells, and the 165-residue mature protein subunit. VEGF-165 binds strongly to heparin for which the strongly basic sequence between residues 115-159 is thought to be responsible (Fig. 1) (Thomas, J. Biol. Chem., 271:603-06 (1996)). The other members of the VEGF family are homodimeric proteins with shorter or longer subunits of 121, 189 and 206 residues (VEGF-121, VEGF-189, and VEGF-206, respectively) (Tischer et al., J. Biol. Chem. 266:11947-54, 1991; Park et al., Mol Biol Cell 4:1317-26 (1993)). The 4 forms of VEGF arise from alternative splicing of up to 8 exons of the VEGF gene (VEGF-121, exons 1-5,8; VEGF-165, exons 1-5,7,8; VEGF-189, exons 1-5, 6a, 7, 8; VEGF-206, exons 1-5, 6b, 7, 8 (exon 6a and 6b refer to 2 alternatively spliced forms of the same exon)) (Houck et al., Mol. Endocr., 5:1806-14 (1991)). The VEGF sequences contain eight conserved disulfide-forming core cysteine residues. All VEGF genes encode signal peptides that direct the protein into the secretory pathway. However, only VEGF-121 and -165 are found to be readily secreted by cultured cells whereas VEGF-189 and -206 remain associated with the extracellular matrix. These VEGF forms possess an additional highly basic sequence, corresponding to residues 115-139 in VEGF-189 and -206 (matrix-targeting sequence), which confers high affinity to acidic components of the extracellular matrix (Thomas, J. Biol. Chem. 271:603-06 (1996)).

Mitogenic activity of the various VEGF isoforms varies depending on each isoform. For example, VEGF-121 and VEGF-165 have very similar mitogenic activity for endothelial cells. However, VEGF-189 and VEGF-206 are only weakly mitogenic (Ferrara et al., Endocr. Rev. 13:18-32, 1992). The reduced activity of these isoforms is attributed to their strong association with cells and matrix, as evidenced by the normal mitogenic activity of a mutant of VEGF-206 which lacks the 24-residue "matrix targeting" sequence common to VEGF-189 and VEGF-206 (residues 115-139 in Fig. 1) (Ferrara et al., Endocr. Rev. 13:18-32, 1992).

An N-terminal fragment of VEGF-165 generated by plasmin (VEGF (1-110)) bound with the same affinity to the KDR receptor as VEGF-165 and VEGF-121 whereas the C-terminal VEGF-fragment (111-165) had no binding activity (Keyt et al., J. Biol. Chem. 271:7788-95, 1996). Interestingly, in this study the mitogenic activity of VEGF-121 and VEGF-110 was reduced by approximately 110-fold as compared to VEGF-165, suggesting a potential role of the C-terminal domain of VEGF-165 in the biological potency of VEGF isoforms. The significance of this finding is somewhat unclear in view of earlier results showing the equivalent potency of VEGF-121 and VEGF-165 on endothelial cell growth. Furthermore, since functional interaction of VEGF with the KDR receptor is thought to be dependent at least in part on cell surface heparin sulfate proteoglycan(s) (Cohen et al., J. Biol. Chem., 270:11322-26, 1995; Tessler et al., J. Biol. Chem. 269:12456-61; 1994) it is conceivable that differences in results arise from differences in various experimental systems. In this context it is unclear to what extent cell surface heparin sulfates regulate the functional interaction of VEGF-121 (lacking a heparin-binding domain) and VEGF-165 (possessing a heparin-binding domain) (Tessler et al., J. Biol. Chem. 269:12456-61, 1994; Cohen et al., J. Biol. Chem. 270:11322-26, 1995; Gitay-Goren et al., J. Biol. Chem. 271:5519-23 (1996)).

VEGFs are related to platelet-derived growth factor (PDGF)(Andersson et al., Growth Factors 12:159-64, 1995). VEGFs are also related to the family of proteins derived from the Placenta Growth Factor (PlGF) gene, PlGF-129 and PlGF-150 (Maglione et al., Proc. Natl. Acad. Sci. USA 88:9267-71, 1991; Oncogene 8:925-31, 1993). More recently several additional VEGF-related genes have been identified and termed VEGF-B (also called VEGF-related factor VRF-1) (Grimmond et al., Genome Res. 6:122-29, 1996; Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81, 1996) VRF-2 (Grimmond et al., Genome Res. 6:122-29, 1996), and VEGF-C (Joukov et al., EMBO J. 15:290-98, 1996; Lee et al., Proc. Natl. Acad. Sci. USA 93:1988-92, 1996) and VEGF-3 (PCT Application No. PCT/US95/07283, published on December 12, 1996 as WO96/39421). Finally, two virally encoded VEGF-related sequences have been identified, poxvirus ORF-1 and ORF-2 (Lyttle et al., J. Virol. 68:84-92, 1994). With the exception of PDGF, these proteins are referred to as VEGF-related proteins [VRPs]. Sequences of examples of VRPs are depicted in Figure 1.

The VRPs, and the PDGFs known so far have 8 cysteines within their sequences that are relatively positionally conserved. The protein sequence spanning the conserved cysteines is therefore referred to herein as the core sequence, and the first N-terminal conserved cysteine of the sequence is referred to herein as the "First cysteine of the core sequence" or "first core cysteine."

Interestingly, members of the VEGF families can form heterodimers, such as heterodimers consisting of VEGF and PlGF subunits (DiSalvo et al., J. Biol. Chem. 270:7717-23, 1995; Cao et al., J. Biol. Chem. 271: 3154-62, 1996). Whereas VEGFs are highly potent in stimulating angiogenesis and endothelial cell proliferation, VEGF/PlGF heterodimers are less potent mitogens, and PlGF homodimers have little or no mitogenic activity (DiSalvo et al., J. Biol. Chem. 270:7717-23, 1995; Cao et al.,

J. Biol. Chem. 271: 3154-62, 1996). In other experiments, VEGF-165/VEGF-B heterodimers were found to form after transfection of cells with both genes (Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81, 1996).

VEGFs interact with two receptors present on endothelial cells, KDR/flk-1 (Terman et al., Biochem. Biophys. Res. Commun. 187:1579-86, 1992), and flt-1 (De Vries et al., Science 255:989-91, 1992). Systematic site-directed mutagenesis of VEGF-165 by alanine scanning of charged residues has shown that residues D63, E64 and E67 are involved in binding of VEGF to flt-1 whereas the basic residues R82, KI84, and H86 contribute strongly to binding to KDR (Keyt et al., J. Biol. Chem. 271:5638-46, 1996).

VRPs are known to bind to one or more of three different endothelial cell receptors, each of which is a single transmembrane protein with a large extracellular portion comprised of 7 immunoglobulin-type domains and a cytoplasmic portion that functions as a tyrosine kinase. These receptors are KDR/flk-1 (Terman et al., Biochem. Biophys. Res. Commun. 187:1579-86, 1992), flt-1 (De Vries et al., Science 255:989-91, 1992), and flt-4 (Pajusola et al., Cancer Res. 52:5738-43, 1992). There are distinct selectivities between these receptors and the various VEGF ligands that have not been completely elucidated as yet. However, it is known that VEGF binds to KDR and flt1 (Terman et al., Growth Factors 11:187-95, 1994) but not flt4 (Joukov et al., EMBO J. 15:290-98, 1996), PlGF binds to flt 1 but not KDR (Terman et al., Growth Factors 11:187-95, 1994) and flt4 (Joukov et al., EMBO J. 15:290-98, 1996), VEGF-C binds to flt-4 (Joukov et al., EMBO J. 15:290-98, 1996) but it is controversial whether it also binds to KDR (Joukov et al., EMBO J. 15:290-98, 1996; Lee et al., Proc. Natl. Acad. Sci. USA 93:1988-92, 1996). The receptor specificity for VEGF-B/VRF-1, VRF-2 and the virally encoded VRPs is not presently known. However, since VEGF-B stimulates

endothelial cell proliferation (Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81, 1996) it may be speculated that VEGF-B can bind to KDR because KDR is thought to be primarily responsible for the angiogenic response of endothelial cells to VEGF-like growth factors (Gitay-Goren et al., J. Biol. Chem. 271:5519-23 (1996)).

Most of the VRPs have been shown to activate the KDR receptor which is thought to make endothelial cells "angiogenesis-competent." Evidence for such activity has been presented for VEGF-B which stimulates endothelial cell proliferation (Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81, 1996), VEGF-C which stimulates endothelial cell migration and proliferation (Joukov et al., EMBO J. 15:290-98, 1996; Lee et al., Proc. Natl. Acad. Sci. USA 93:1988-92, 1996), and both known virally encoded VRPs which were reported to be angiogenic (Lyttle et al., J. Virol. 68:84-92, 1994). A notable exception are PlGF isoform homodimers which have negligible mitogenic activity for endothelial cells. However, PlGF/VEGF heterodimers still retain considerable mitogenic activity (DiSalvo et al., J. Biol. Chem. 270:7717-23, 1995; Cao et al., J. Biol. Chem. 271: 3154-62, 1996).

VEGFs are expressed in many different tissues. Similarly, VRP genes are also expressed in multiple tissues but it is of particular interest that VEGF-B and to a lesser extent VRF-2 are strongly expressed in human heart and skeletal muscle (Grimmond et al., Genome Res. 6:122-29, 1996; Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81, 1996). In fact, VEGF-B is expressed considerably more strongly in mouse heart tissue than VEGF (Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81, 1996). VEGF-C is also strongly expressed in several human tissues, most notably in heart and skeletal muscle (Joukov et al., EMBO J. 15:290-98, 1996). This expression pattern, and the exquisite specificity of VRPs for endothelial cells, suggest that these factors play a

physiological role in angiogenesis in these tissues. This is thought to be relevant in pathological situations such as coronary ischemia where collateral angiogenesis is required to provide the heart muscle with an adequate capillary blood vessel supply. It has been shown that transient ischemia induced by coronary artery ligation or hypoxia rapidly upregulates VEGF mRNA in the rat or pig heart in vivo and hypoxia induces VEGF mRNA in cardiac myocytes and smooth muscle cells in vitro (Hashimoto et al., Am. J. Physiol 267, H1948-H1954, 1994; Banai, et al., Cardiovac. Res. 28:1176-79, 1994; Circulation 90, 649-52, 1994). The strong expression of VEGF and VRPs in the heart may help to ensure a redundant and competent regulatory system capable of inducing new blood vessel formation when it is needed. Collateral blood vessel formation is also required in peripheral (lower limb) vascular ischemias and in cerebral ischemias (stroke). Finally, new blood vessel formation is required in tissue repair after wounding. In this context, it is worth noting that VEGF is upregulated in epidermal keratinocytes during skin wound healing (Brown et al., J. Exp. Med. 176:1375-79, 1992). Thus, therapy of various ischemic conditions such as cardiac infarction, chronic coronary ischemia, chronic lower limb ischemia, wound healing and stroke with VRPs may be potentially clinically beneficial.

Summary Of The Invention

The present invention is directed to novel truncated forms of VEGF-related proteins (VRPs), preferably human VRPs. The preferred use of the truncated VRPs and nucleic acid molecule compositions of the invention is to use such compositions to aid in the treatment of patients with heart disease, wounds, or other ischemic conditions by stimulating angiogenesis in such patients. The amino acid sequences of VRPs include eight disulfide-forming cysteine residues that are conserved between

VRPs and VEGF proteins (core cysteines). VRPs include, but are not limited to, VEGF-B, VEGF-C, VRF-2, ORF-1, ORF-2, and PlGFs.

A first aspect of the invention provides for a truncated VRP having a deletion of at least one of the amino acid residues N-terminal to the first cysteine of the core sequence of said subunit. Such compositions would be substantially free of other proteins. Preferably, the truncations range from truncating minimally the N-terminal residue of the mature protein subunit only(not including the signal sequence) and maximally all N-terminal amino acids of the mature protein up to and including the residue N-terminal to (prior to) the first core cysteine residue. In more preferred aspects, all of the amino acid residues N-terminal to the first cysteine of the core sequence, except the 1 to 5 amino acid residues immediately N-terminal to said first cysteine, are deleted.

Although the amino acid deletions may consist of deletions of non-adjacent amino acid residues in the N-terminal sequence, it is preferred that the deletions be of consecutive amino acid residues. Thus, in one preferred aspect, the invention comprises human VRPs that have deletions of amino acid residue sequences of increasing lengths from the N-terminus of the N-terminal sequence up to the first cysteine of the core sequence of the VRP subunit sequence.

In preferred aspects, the invention provides for truncated versions of the VRPs VEGF-B, VRF-2, VEGF-C, VEGF-3, PlGF, poxvirus ORF-1, and poxvirus ORF-2. In such truncated VRPs, each subunit may independently have a deletion of at least one of the amino acid residues N-terminal to the first cysteine of the core sequence of said subunit, or only one of the subunits may have such a deletion.

In particular embodiments, the truncated VRP subunit comprises a VRP subunit wherein various numbers of amino acid residues N-terminal to the first cysteine of the core sequence are deleted. In one aspect, the remaining N-terminal residues

consist of consecutive amino acid residues derived from the N-terminal sequence. These consecutive N-terminal residues may be derived from any location in the N-terminal sequence, however, a consecutive sequence starting from the N-terminus of the N-terminal sequence is preferred, and a sequence consisting of consecutive amino acid residues immediately N-terminal to the first cysteine of the core sequence of the VRP subunit is most preferred. Examples of such most preferred embodiments are depicted in Figure 2.

In other embodiments, the amino acid residues N-terminal to the first cysteine of the core sequence of the truncated VRPs of the invention are a randomly selected amino acid sequence, in yet other embodiments, these amino acid residues are derived from the N-terminal sequence of the full length VRP sequence, but are not necessarily consecutive amino acids from the full length VRP sequence.

Thus, in one most preferred aspect, the invention provides a truncated VRP subunit wherein the amino acid residues N-terminal to the first cysteine of the core sequence of said subunit are deleted.

In other aspects, the invention provides a truncated VRP subunit wherein the amino acid sequence N-terminal to the core sequence comprises 11 to 20, more preferably 11 to 15, more preferably 6 to 10, and most preferably 2 to 5 amino acid residues.

Preferably, the amino acid sequence N-terminal to the core sequence comprises the consecutive amino acid residues immediately N-terminal to the first cysteine of the core sequence of said VRP subunit. Thus, in these preferred embodiments, the truncated VRP comprises the core sequence, the necessary C-terminal sequence to the core sequence, and further comprises at the region N-terminal to the first cysteine of the core sequence, the 11 to 20, more preferably the 11 to 15, more preferably the 6 to 10, and most preferably the 2 to 5

consecutive amino acid residues of the amino acid sequence that is immediately N-terminal to the first cysteine of the core sequence of the full length VRP sequence.

Those skilled in the art will recognize that where a truncated VRP subunit comprises, for example, (X) amino acids N-terminal to the first cysteine of the core sequence, that such a truncated VRP subunit is one where the corresponding full length VRP subunit comprises (X + 1) amino acids N-terminal to the first cysteine of the core sequence.

The truncated VRPs of the invention include truncated VRP homodimers comprising two truncated VRP subunits of the invention, wherein the two truncated VRP subunits have the same amino acid sequence, and also include truncated VRP heterodimers comprising two truncated VRP subunits of the invention wherein the two subunits have different amino acid sequences from each other.

For purposes of the present invention, the term "first N-NN" amino acids where N and NN each represent numbers of amino acids, for example, the first 10-15 amino acids, denotes the first N-NN amino acids (e.g., the first 10-15 amino acids) after the signal peptide sequence of the designated VRP. The term N-NN encompasses a deletion of anywhere from N to NN of the first amino acids after the signal sequence. Thus, in more preferred aspects, the truncated VRP subunit comprises a truncated hVEGFB protein subunit wherein the first 10-15 amino acids are deleted; more preferably, the first 15-20 amino acids are deleted; more preferably, the first 20-25 amino acids are deleted; and most preferably, the first 23-24 amino acids are deleted.

In other more preferred aspects, the truncated VRP subunit comprises a truncated hVRF2 protein subunit wherein the first 10-15 amino acids are deleted; more preferably, the first 15-20 amino acids are deleted; more preferably, the first 20-25 amino

acids are deleted; and most preferably, the first 23-24 amino acids are deleted.

In other more preferred aspects, the truncated VRP subunit comprises a truncated hVEGFC protein subunit wherein the first 95-100 amino acids are deleted; more preferably, the first 100-105 amino acids are deleted; more preferably, the first 105-110 amino acids are deleted; and most preferably, the first 108-109 amino acids are deleted.

In other more preferred aspects, the truncated VRP subunit comprises a truncated hPlGF protein subunit wherein the first 16-21 amino acids are deleted; more preferably, the first 21-26 amino acids are deleted; more preferably, the first 26-31 amino acids are deleted; and most preferably, the first 29-30 amino acids are deleted.

In other more preferred aspects, the truncated VRP subunit comprises a truncated hVEGF3 protein subunit wherein the first 10-15 amino acids are deleted; more preferably, the first 15-20 amino acids are deleted; more preferably, the first 20-25 amino acids are deleted; and most preferably, the first 23-24 amino acids are deleted.

In other more preferred aspects, the truncated VRP subunit comprises a truncated pvORF1 protein subunit wherein the first 20-25 amino acids are deleted; more preferably, the first 25-30 amino acids are deleted; more preferably, the first 30-35 amino acids are deleted; and most preferably, the first 33-34 amino acids are deleted.

In other more preferred aspects, the truncated VRP subunit comprises a truncated pvORF2 protein subunit wherein the first 30-35 amino acids are deleted; more preferably, the first 35-40 amino acids are deleted; more preferably, the first 40-45 amino acids are deleted; and most preferably, the first 43-44 amino acids are deleted. The sequences of some exemplary preferred truncated VRP subunits are set out in Figure 2.

The invention also provides for nucleic acid molecules coding for the truncated VRP subunits described herein. The nucleic acid molecules may be, for example, DNA, cDNA or RNA. The invention also provides for recombinant DNA vectors comprising the nucleic acid molecules encoding the truncated VRPs, and host cells transformed with such recombinant DNA vectors, wherein such vectors direct the synthesis of a truncated VRP subunit such as those described herein.

The invention further provides for nucleic acid molecules encoding biosynthetic precursor forms of N-terminally truncated subunits of VRPs for the purpose of facilitating the expression in suitable host systems. Such nucleic acid molecules are comprised of DNA encoding a signal peptide that precedes the truncated subunits at their N-termini. The signal sequences of VEGF or VRPs would be used to construct appropriate signal peptide-containing truncated forms of VRPs. The human VEGF signal peptide is as follows:

mnfllswvhwslalllylhhakwsqa (I) -- [SEQ I.D. NO. 40] --

Alternatively, the signal peptides shown in Figure 1 may be used. Preferably, the signal peptide specific for the truncated VRP is used in the construct.

In order to facilitate signal peptide cleavage in mammalian cells after fusion of the signal sequence to truncated forms of VRP, it may be necessary to include the first or the first two residues of the mature VRP peptide sequence, e.g. proline (P), or proline-valine (PV) for hVEGFB. Thus, an appropriate nucleic acid molecule would be comprised of DNA encoding the signal sequence of VEGF-B, optionally followed by a codon for proline (the first residue of mature VEGF-B), optionally followed by a codon for valine (the second residue of mature VEGF-B), and followed by DNA encoding the N-terminally truncated VEGF-B. The invention also provides for other appropriate signal peptide fusion constructs. best suitable for non-mammalian hosts, as known by those skilled in

the art. Those skilled in the art will recognize that the signal peptides should optionally include residues needed for facilitation of signal peptide cleavage in mammalian cells for the various truncated VRP subunits of the present invention.

Thus, the present invention provides for recombinant DNA expression vectors wherein the 5' end of the nucleic acid molecule coding for the truncated VRP subunit is operably linked to a DNA sequence that codes for a signal peptide. The signal peptide may be a human VRP signal peptide. Moreover, the DNA sequence coding for said signal peptide may be operably linked at the 3' end of said DNA sequence to DNA coding for the first amino acid residue of the mature non-truncated VRP subunit, and wherein the 3' end of said DNA coding for said residue is operably linked to the nucleic acid molecule coding for said truncated VRP subunit. In other aspects, the DNA sequence coding for said signal peptide is operably linked at the 3' end of said DNA sequence to DNA coding for the first two amino acid residues of the mature non-truncated VRP subunits, and wherein the 3' end of said DNA coding for said two residues is operably linked to said nucleic acid molecule coding for said truncated VRP subunit. Thus, in preferred aspects, the invention also provides a truncated VRP subunit of the invention as described above, further comprising at the N-terminus of said truncated VRP subunit, the first one or two amino acid residues of the mature non-truncated VRP subunit. Those skilled in the art will recognize that such truncated VRP subunits of the invention include those wherein the final number of amino acids N-terminal to the first cysteine of the core sequence (including the additional one or two amino acids that may facilitate signal peptide cleavage) is at least one less than the number of amino acids N-terminal to the first cysteine of the core sequence of the corresponding full length VRP.

In other preferred aspects, the invention provides truncated VRP homodimers or heterodimers comprising two truncated VRP subunits wherein said truncated VRP subunits comprise at the N-terminus of said truncated VRP subunits, the first one or two amino acid residues of the mature non-truncated VRP subunit.

In preferred aspects, the recombinant nucleic acid molecule coding for a truncated VRP subunit of the invention is operably linked to control sequences operable in a host cell transformed with said vector. The present invention also provides for transformed or transfected host cells comprising the recombinant DNA vectors of the invention.

The present invention also includes delivery vectors which comprise nucleic acid molecules coding for the truncated VRPs of the invention. Such delivery vectors may be, for example, viral vectors. Such viral vectors may be, for example, adenovirus vectors or adenovirus-associated virus vectors. In other aspects of the invention are provided an adenovirus vector comprising a nucleic acid molecule coding for a truncated VRP of the invention operably linked at the 5' end of the nucleic acid molecule to a DNA sequence that codes for a signal peptide. Preferably, the signal peptide is selected from the group consisting of VEGF signal peptide, VEGF-B signal peptide, VRF-2 signal peptide, VEGF-C signal peptide, PlGF signal peptide, VEGF-3 signal peptide, poxvirus ORF-1 signal peptide, and poxvirus ORF-2 signal peptide. Preferably said signal peptide is VEGF-B signal peptide. In preferred aspects, the DNA sequence coding for the signal peptide is operably linked at the 3' end of the DNA sequence to DNA coding for the first amino acid residue of the mature non-truncated VRP subunit, and wherein the 3' end of said DNA coding for said residue is operably linked to the nucleic acid molecule coding for said truncated VRPs. In most preferred aspects, the

adenovirus vector comprises a nucleic acid molecule which codes for a truncated VRP subunit of Figure 2.

In further preferred aspects of the invention are provided a filtered-injectable adenovirus vector preparation comprising a recombinant adenoviral vector, said vector containing no wild-type virus and comprising: a partial adenoviral sequence from which the E1A/E1B genes have been deleted, and a transgene coding for a truncated VRP subunit, driven by a promoter flanked by the partial adenovirus sequence; and a pharmaceutically acceptable carrier. In preferred aspects, the preparation has been filtered through a 30 micron filter. In other preferred aspects the truncated VEGF subunit is a truncated VEGF subunit of Figure 2. In another preferred aspect, the injectable adenoviral vector preparation comprises a promoter selected from the group consisting of a CMV promoter, a ventricular myocyte-specific promoter, and a myosin heavy chain promoter.

In other aspects, the invention provides a method of producing a truncated VRP polypeptide comprising growing, under suitable conditions, a host cell transformed or transfected with the recombinant DNA expression vector of the invention in a manner allowing expression of said polypeptide, and isolating said polypeptide from the host cell. Suitable conditions are then provided for the truncated VRP peptide to fold into a truncated VRP subunit. In mammalian cells, such conditions should be naturally provided by the cell. In non-mammalian cells, appropriate pH, isotonicity, and reducing conditions must be provided, such as those described in, for example, Example 2. Most preferably, the invention provides a method of producing a truncated VRP wherein suitable conditions are provided for said truncated VRP subunit to dimerize with a second VRP subunit selected from the group consisting of VRP subunits and truncated VRP subunits. In preferred aspects of the invention are provided methods of producing a truncated VRP

homodimer comprising two truncated VRP subunits having the same amino acid sequence.

In other aspects of the invention are provided methods of producing truncated VRP heterodimers wherein the two VRP subunits have different amino acid sequences. Such heterodimers may consist of one truncated VRP subunit and one non-truncated VRP subunit, or both VRP subunits may be truncated. The two subunits may be derived from different VRPs. For example, the heterodimer may consist of one VEGF-B subunit and one truncated VEGF-C subunit, or both subunits may be truncated.

In further preferred aspects, the present invention provides pharmaceutical compositions comprising a truncated VRP subunit of the present invention, in a suitable carrier. The invention includes methods of stimulating blood vessel formation comprising administering to a patient such a pharmaceutical composition.

Methods are provided using the compounds of the present invention to stimulate endothelial cell growth or endothelial cell migration in vitro comprising treating said endothelial cells with truncated VRPs.

The present invention also provides methods of treating a patient suffering from a heart disease comprising administering to said patient a nucleic acid molecule coding for at least one truncated VRP subunit, said nucleic acid molecule capable of expressing the truncated VRP subunit in said patient. In an additional embodiment, methods are provided of stimulating angiogenesis in a patient comprising administering a therapeutic amount of a pharmaceutical composition comprising a truncated VRP of the present invention.

Preferably, the pharmaceutical composition is in a therapeutically suitable delivery system. In other preferred aspects, a potentiating agent is administered to potentiate the angiogenic effect of said truncated VRP. Such agents include,

for example, basic Fibroblast Growth Factor (bFGF) (FGF-2), acidic FGF (aFGF) (FGF-1), FGF-4, FGF-5, FGF-6, or any FGF or other angiogenic factor that stimulates endothelial cells. Thus, in one aspect of the invention is provided a pharmaceutical composition comprising a truncated VRP and one or more potentiating agents. The pharmaceutical compositions may also be used to treat patients suffering from ischemic conditions such as cardiac infarction, chronic coronary ischemia, chronic lower limb ischemia, stroke, and peripheral vascular disease. Methods are also provided using the pharmaceutical compositions of the present invention to treat wounds, such as dermal or intestinal wounds.

In preferred embodiments, methods are provided of stimulating angiogenesis in a patient comprising delivering a delivery vector to the myocardium of the patient by intracoronary injection directly into one or both coronary arteries, said vector comprising a nucleic acid molecule coding for at least one truncated VRP subunit, wherein said vector is capable of expressing the truncated VRP subunit in the myocardium.

In other preferred embodiments, the method may be used for stimulating coronary collateral vessel development.

In more preferred embodiments, a method is provided for stimulating vessel development in a patient having peripheral vascular disease, comprising delivering a delivery vector to the peripheral vascular system of the patient by intra-femoral artery injection directly into one or both femoral arteries, said vector comprising a transgene coding for a truncated VRP subunit, and capable of expressing the truncated VRP subunit in the peripheral vascular system, thereby promoting peripheral vascular development.

Preferably the delivery vector used in the invention is a viral delivery vector. In one preferred aspect, the delivery vector is a replication-deficient adenovirus vector. In

another preferred aspect, the delivery vector is an adeno-associated virus vector.

Brief Description Of The Drawings

Figure 1 depicts the amino acid sequences of VEGF-B [SEQ I.D. NO. 1], VRF-2 [SEQ I.D. NO. 2], VEGF-C [SEQ I.D. NO. 3], PlGF (human PlGF-2) [SEQ I.D. NO. 4], VEGF-3 [SEQ I.D. NO. 5], poxvirus ORF-1 [SEQ I.D. NO. 6], and poxvirus ORF-2 [SEQ I.D. NO. 7]. Lower case letters signify signal peptides that are cleaved from the mature protein. The eight cysteines of the core sequence are underlined. Sequences are described in the following references: human VEGF-B: Grimmond et al., Genome Res. 6:122-29 (1996); Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81 (1996); mouse VEGF-B: Olofsson et al., Proc. Natl. Acad. Sci. U.S.A. 93:2567-81 (1996); human VRF-2: Grimmond et al., Genome Res. 6:122-29 (1996); human VEGF-C: Joukov et al., EMBO J. 15:290-98 (1996); Lee et al., Proc. Natl. Acad. Sci. USA 93:1988-92 (1996); PlGF: Maglione et al., Oncogene 8:925-31 (1993); Hauser & Weich, Growth Factors 9:259-68 (1993); human VEGF3: PCT Application Serial No. PCT/US95/07283, published on December 12,, 1996, as WO96/39421; poxvirus ORF-1 and ORF-2: Lyttle et al., J. Virol. 68:84-92 (1994).

Figure 2a-2f depicts examples of truncated VRP amino acid sequences below the corresponding full length (F/L) VRP sequence. The amino acid sequences of each truncation are listed as follows:

2a(F/L)[SEQ I.D. NO. 34](1) [SEQ I.D. NO. 8]; 2a(2) [SEQ I.D. NO. 9]; 2a(3) [SEQ I.D. NO. 10]; 2a(4) [SEQ I.D. NO. 11]; 2a(5) [SEQ I.D. NO. 12]; 2a(6) [SEQ I.D. NO. 13]; 2b (F/L) [SEQ I.D. NO. 35]; (1) [SEQ I.D. NO. 14]; 2b(2) [SEQ I.D. NO. 15]; 2b(3) [SEQ I.D. NO. 16]; 2b(4) [SEQ I.D. NO. 17]; 2c(F/L) [SEQ I.D. NO. 36]; (1) [SEQ I.D. NO. 18];

2c(2) [SEQ I.D. NO. 19]; 2c(3) [SEQ I.D. NO. 20]; 2c(4) [SEQ I.D. NO. 21]; 2d(F/L) [SEQ I.D. NO. 37]; (1) [SEQ I.D. NO. 22]; 2d(2) [SEQ I.D. NO. 23]; 2d(3) [SEQ I.D. NO. 24]; 2d(4) [SEQ I.D. NO. 25]; 2e(F/L [SEQ I.D. NO. 38] (1) [SEQ I.D. NO. 26]; 2e(2) [SEQ I.D. NO. 27]; 2e(3) [SEQ I.D. NO. 28]; 2e(4) [SEQ I.D. NO. 29]; 2f(F/L) [SEQ I.D. NO. 39]; (1) [SEQ I.D. NO. 30]; 2f(2) [SEQ I.D. NO. 31]; 2f(3) [SEQ I.D. NO. 32]; and 2f(4) [SEQ I.D. NO. 33].

Detailed Description Of The Invention

Construction of Novel Truncated VRP Sequences

In a first aspect the invention features a truncated VRP comprising at least one truncated VRP subunit. By "truncated VRP subunit" it is meant a VRP subunit having an amino acid sequence substantially similar to one of the VRPs, for example, but not limited to, one of the sequences shown in Figure 1, or an analog or derivative thereof, wherein at least one of the N-terminal amino-acid residues N-terminal to the first cysteine of the core sequence of the mature subunit is deleted. A sequence that is "substantially similar" to a VRP comprises an amino acid sequence that is at least 25% homologous to the 8 cysteine core sequence of VEGF-B, comprises all of the essential conserved cysteine residues of said core sequence, and retains VRP activity. By "truncated VRP subunit" is also meant a VRP subunit wherein at least one of the N-terminal amino acid residues N-terminal to the first cysteine of the VEGF core sequence is deleted, and, at least one of the cysteines of the core sequence is deleted, wherein said cysteine is non-essential. A non-essential cysteine is one that is not required to retain VRP activity. Such non-essential cysteines have been described in connection with PDGF. (Potgens, et al. J. Biol. Chem. 269:32879-85 (1994)).

By "identity" is meant a property of sequences that measures their similarity or relationship. Identity is

measured by dividing the number of identical residues by the total number of residues and multiplying the product by 100. Thus, two copies of exactly the same sequence have 100% identity, but sequences that are less highly conserved and have deletions, additions, or replacements may have a lower degree of identity. In calculating sequence identity, the two sequences are compared starting at the carboxy terminus of the N-terminal deletion. Those skilled in the art will recognize that several computer programs are available for determining sequence identity.

Analogs of a truncated VRP polypeptide or subunit are functional equivalents having similar amino acid sequence and retaining, to some extent, one or more activities of the related truncated VRP polypeptide or subunit. By "functional equivalent" is meant the analog has an activity that can be substituted for one or more activities of a particular truncated VRP polypeptide or subunit. Preferred functional equivalents retain all of the activities of a particular truncated VRP polypeptide or subunit, however, the functional equivalent may have an activity that, when measured quantitatively, is stronger or weaker, as measured in VRP functional assays, for example, such as those disclosed herein. In most cases, such truncated VRP polypeptides or subunits must be incorporated into a truncated VRP dimer in order to measure functional activity. Preferred functional equivalents have activities that are within 1% to 10,000% of the activity of the related truncated VRP polypeptide or subunit, more preferably between 10% to 1000%, and more preferably within 50% to 200%.

The ability of a derivative to retain some activity can be measured using techniques described herein. Derivatives include modification occurring during or after translation, for example, by phosphorylation, glycosylation, crosslinking, acylation, proteolytic cleavage, linkage to an antibody

molecule, membrane molecule or other ligand (see Ferguson *et al.*, 1988, *Annu. Rev. Biochem.* 57:285-320).

Specific types of derivatives or analogs also include amino acid alterations such as deletions, substitutions, additions, and amino acid modifications. A "deletion" refers to the absence of one or more amino acid residue(s) in the related polypeptide. An "addition" refers to the presence of one or more amino acid residue(s) in the related polypeptide. Additions and deletions to a polypeptide may be at the amino terminus, the carboxy terminus, and/or internal. Amino acid "modification" refers to the alteration of a naturally occurring amino acid to produce a non-naturally occurring amino acid. A "substitution" refers to the replacement of one or more amino acid residue(s) by another amino acid residue(s) in the polypeptide. Derivatives can contain different combinations of alterations including more than one alteration and different types of alterations.

While the effect of an amino acid change on VRP activity varies depending upon factors such as phosphorylation, glycosylation, intra-chain linkages, tertiary structure, and the role of the amino acid in the active site or a possible allosteric site, it is generally preferred that the substituted amino acid is from the same group as the amino acid being replaced. To some extent the following groups contain amino acids which are interchangeable: the basic amino acids lysine, arginine, and histidine; the acidic amino acids aspartic and glutamic acids; the neutral polar amino acids serine, threonine, cysteine, glutamine, asparagine and, to a lesser extent, methionine; the nonpolar aliphatic amino acids glycine, alanine, valine, isoleucine, and leucine (however, because of size, glycine and alanine are more closely related and valine, isoleucine and leucine are more closely related); and the aromatic amino acids phenylalanine, tryptophan, and tyrosine. In addition, although classified in different categories,

alanine, glycine, and serine seem to be interchangeable to some extent, and cysteine additionally fits into this group, or may be classified with the polar neutral amino acids.

Preferred derivatives have one or more amino acid alteration(s) which do not significantly affect the activity of the related truncated VRP polypeptide or subunit. In regions of the truncated VRP polypeptide or subunit not necessary for VRP activity, amino acids may be deleted, added or substituted with less risk of affecting activity. In regions required for VRP activity, amino acid alterations are less preferred as there is a greater risk of affecting VRP activity. Such alterations should be conservative alterations. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent.

Conserved regions tend to be more important for protein activity than non-conserved regions. Standard procedures can be used to determine the conserved and non-conserved regions important for VRP activity using *in vitro* mutagenesis techniques or deletion analyses and measuring VRP activity as described by the present disclosure.

Derivatives can be produced using standard chemical techniques and recombinant nucleic acid molecule techniques. Modifications to a specific polypeptide may be deliberate, as through site-directed mutagenesis and amino acid substitution during solid-phase synthesis, or may be accidental such as through mutations in hosts which produce the polypeptide. Polypeptides including derivatives can be obtained using standard techniques such as those described in Sambrook *et al.*, *Molecular Cloning*, Cold Spring Harbor Laboratory Press (1989). For example, Chapter 15 of Sambrook describes procedures for site-directed mutagenesis of cloned DNA.

By a "truncated VRP polypeptide" is meant a polypeptide comprising the amino acid sequence of a truncated VRP subunit

of the invention, or a functional analog or derivative thereof as described herein. The term "truncated VRP polypeptide" also includes a truncated VRP subunit; the term subunit generally referring to a peptide that has been folded into an active three-dimensional structure.

By "truncated VRP" is meant a dimer of two VRP subunits. The two subunits may be derived from two different VRPs where both subunits are truncated VRP subunits. One or both of the subunits may be truncated; the two subunits may also have different N-terminal deletions.

It is advantageous that the truncated VRP, truncated VRP subunit, or truncated VRP polypeptide be enriched or purified. By the use of the term "enriched" in this context is meant that the specific amino acid sequence constitutes a significantly higher fraction (2 - 5 fold) of the total of amino acid sequences present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other amino acid sequences present, or by a preferential increase in the amount of the specific amino acid sequence of interest, or by a combination of the two. However, it should be noted that enriched does not imply that there are no other amino acid sequences present, just that the relative amount of the sequence of interest has been significantly increased. The term "significant" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other amino acid sequences of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no amino acid sequence from other sources. The other source amino acid sequence may, for example, comprise amino acid encoded by a yeast or bacterial genome, or a cloning vector such as pUC19. The term is meant to cover only those

situations in which man has intervened to elevate the proportion of the desired amino acid sequence.

It is also advantageous for some purposes that an amino acid sequence be in purified form. The term "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 10 fold greater, *e.g.*, in terms of mg/ml). Purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. The substance is preferably free of contamination at a functionally significant level, for example 90%, 95%, or 99% pure.

In another aspect the invention features a nucleic acid molecule encoding a truncated VRP polypeptide or subunit.

In some situations it is desirable for such nucleic acid molecule to be enriched or purified. By the use of the term "enriched" in reference to nucleic acid molecule is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2 - 5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that enriched does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased. The term significant here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other nucleic acids of about at least 2 fold, more preferably at least 5 to 10 fold or

even more. The term also does not imply that there is no DNA or RNA from other sources. The other source DNA may, for example, comprise DNA from a yeast or bacterial genome, or a cloning vector such as pUC19. This term distinguishes from naturally occurring events, such as viral infection, or tumor type growths, in which the level of one mRNA may be naturally increased relative to other species of mRNA. That is, the term is meant to cover only those situations in which a person has intervened to elevate the proportion of the desired nucleic acid.

It is also advantageous for some purposes that a nucleotide sequence be in purified form. The term "purified" in reference to nucleic acid molecule does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/ml).

The nucleic acid molecule may be constructed from an existing VRP nucleotide sequence by modification using, for example, oligonucleotide site-directed mutagenesis, or by deleting sequences using restriction enzymes, or as described herein. Standard recombinant techniques for mutagenesis such as *in vitro* site-directed mutagenesis (Hutchinson *et al.*, J. Biol. Chem. 253:6551, (1978), Sambrook *et al.*, Chapter 15, *supra*), use of TAB® linkers (Pharmacia), and PCR-directed mutagenesis can be used to create such mutations. The nucleic acid molecule may also be synthesized by the triester method or by using an automated DNA synthesizer.

The invention also features recombinant DNA vectors and recombinant DNA expression vectors preferably in a cell or an organism. The recombinant DNA vectors may contain a sequence coding for a truncated VRP or a functional derivative thereof in a vector containing a promoter effective to initiate

transcription in a host cell. The recombinant DNA vector can contain a transcriptional initiation region functional in a cell and a transcriptional termination region functional in a cell.

The present invention also relates to a cell or organism that contains the above-described nucleic acid molecule or recombinant DNA vector and thereby is capable of expressing a truncated VRP peptide. The polypeptide may be purified from cells which have been altered to express the polypeptide. A cell is said to be "altered to express a desired polypeptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at lower levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

For example, the entire coding sequence of a truncated VRP subunit or a fragment thereof, may be combined with one or more of the following in an appropriate expression vector to allow

for such expression: (1) an exogenous promoter sequence (2) a ribosome binding site (3) a polyadenylation signal (4) a secretion signal. Modifications can be made in the 5'-untranslated and 3'-untranslated sequences to improve expression in a prokaryotic or eukaryotic cell; or codons may be modified such that while they encode an identical amino acid, that codon may be a preferred codon in the chosen expression system. The use of such preferred codons is described in, for example, Grantham et al., Nuc. Acids Res., 9:43-74 (1981), and Lathe, J. Mol. Biol., 183:1-12 (1985) hereby incorporated by reference herein in their entirety.

If desired, the non-coding region 3' to the genomic VRP sequence may be operably linked to the nucleic acid molecule encoding such VRP subunit. This region may be used in the recombinant DNA vector for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding a VRP gene, the transcriptional termination signals may be provided. Alternatively, a 3' region functional in the host cell may be substituted.

An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. Two DNA sequences (such as a promoter region sequence and a truncated VRP sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation in the coding sequence, (2) interfere with the ability of the promoter region sequence to direct the transcription of a truncated VRP gene sequence, or (3) interfere with the ability of the a truncated VRP gene sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. Thus, to express

a truncated VRP gene, transcriptional and translational signals recognized by an appropriate host are necessary.

Expression and Purification of Novel Truncated VRP Sequences

Examples 2 and 3 describe the expression and purification of novel truncated VRP sequences of the present invention as expressed in baculovirus systems. Those skilled in the art will recognize that the truncated VRPs of the present invention may also be expressed in other cell systems, both prokaryotic and eukaryotic, all of which are within the scope of the present invention. Examples 4-6 provide examples of suitable assays for functional activity of the novel truncated VRPs.

Although the truncated VRPs of the present invention may be expressed in prokaryotic cells, which are generally very efficient and convenient for the production of recombinant proteins, the truncated VRPs produced by such cells will not be glycosylated and therefore may have a shorter half-life in vivo. Prokaryotes most frequently are represented by various strains of E. coli. However, other microbial strains may also be used, including other bacterial strains. Recognized prokaryotic hosts include bacteria such as E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia, and the like. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors may include γgt10, γgt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

To express truncated VRP polypeptides or subunits (or a functional derivative thereof) in a prokaryotic cell, it is

necessary to operably link the truncated VRP sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ, the bla promoter of the β-lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pPR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the trp, recA, λacZ, λacI, and gal promoters of E. coli, the α-amylase (Ulmanen et al., J. Bacteriol. *162*:176-182(1985)) and the ς-28-specific promoters of B. subtilis (Gilman et at., Gene sequence 32:11-20(1984)), the promoters of the bacteriophages of Bacillus (Gryczan, In: The Molecular Biology of the Bacilli, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward et at., Mol. Gen. Genet. *203*:468-478(1986)). Prokaryotic promoters are reviewed by Glick (J. Ind. Microbiot. *1*:277-282(1987)); Cenatiempo (Biochimie *68*:505-516(1986)); and Gottesman (Ann. Rev. Genet. *18*:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold et at. (Ann. Rev. Microbiol. *35*:365-404(1981)). The ribosome binding site and other sequences required for translation initiation are operably linked to the nucleic acid molecule coding for the truncated VRP by, for example, in frame ligation of synthetic oligonucleotides that contain such control sequences. For expression in prokaryotic cells, no signal peptide sequence is required. The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene.

As used herein, "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include

progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. Truncated VRP peptides expressed in prokaryotic cells are expected to comprise a mixture of properly truncated VRP peptides with the N-terminal sequence predicted from the sequence of the expression vector, and truncated VRP peptides which have an N-terminal methionine resulting from inefficient cleaving of the initiation methionine during bacterial expression. Both types of truncated VRP peptides are considered to be within the scope of the present invention as the presence of an N-terminal methionine is not expected to affect biological activity. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Preferred prokaryotic vectors include plasmids such as those capable of replication in E. coil (such as, for example, pBR322, ColEl, pSC101, pACYC 184, πVX. Such plasmids are, for example, disclosed by Sambrook (cf. "Molecular Cloning: A Laboratory Manual", second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, (1989)). Bacillus plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: The Molecular Biology of the Bacilli, Academic Press, NY (1982), pp. 307-329). Suitable Streptomyces plasmids include p1J101 (Kendall et al., J. Bacteriol. *169*:4177-4183 (1987)), and streptomyces bacteriophages such as ϕC31 (Chater et al., In: Sixth International Symposium on Actinomycetales Biology, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). Pseudomonas plasmids are reviewed by John et al. (Rev. Infect. Dis. *8*:693-704(1986)), and Izaki (Jpn. J. Bacteriol. *33*:729-742(1978)).

Eukaryotic host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the truncated VRP peptide. Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either in vivo, or in tissue culture. Mammalian cells which may be useful as hosts include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives.

The truncated VRPs of the present invention may also be expressed in human cells such as human embryo kidney 293EBNA cells which express Epstein-Barr virus nuclear antigen 1, as described, for example, in Olofsson, B. et al., Proc. Natl. Acad. Sci. USA 93:2576-2581 (1996). The cells are transfected with the expression vectors of Example 2 by using calcium phosphate precipitation, and the cells are then incubated for at least 48 hours. The truncated VRP peptides may then be purified from the supernatant as described in Example 3.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences. Another preferred host is an insect cell, for example the Drosophila larvae. Using insect cells as hosts, the Drosophila alcohol dehydrogenase promoter can be used. Rubin, Science *240*:1453-1459(1988).

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals. Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications.

A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides). For a mammalian host, several possible vector systems are available for the expression of truncated VRP peptides.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, cytomegalovirus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

Expression of truncated VRPs in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer et al., J. Mol. Appl. Gen. *1*:273-288(1982)); the TK promoter of Herpes virus (McKnight, Cell *31*:355-365 (1982)); the SV40 early promoter (Benoist et al., Nature (London) 290:304-310(1981)); the yeast gal4 gene sequence promoter (Johnston et al., Proc. Natl. Acad. Sci.

(USA) *79*:6971-6975(1982); Silver et al., Proc. Natl. Acad. Sci. (USA) *81*:5951-5955 (1984)).

Translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes a truncated VRP (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the truncated VRP coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the truncated VRP coding sequence).

A truncated VRP nucleic acid molecule and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a nonreplicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

A vector may be employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be

needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, Molec. Cell. Biol. *3*:280 (1983).

The introduced nucleic acid molecule can be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein et al., Miami Wntr. Symp. *19*:265-274(1982); Broach, In: "The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, Cell *28*:203-204 (1982); Bollon et al., J. Clin. Hematol. Oncol. *10*:39-48 (1980); Maniatis, In: Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608(1980).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, lipofection, calcium phosphate precipitation, direct microinjection, DEAE-dextran

transfection, and the like. The most effective method for transfection of eukaryotic cell lines with plasmid DNA varies with the given cell type. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of truncated VRP or fragments thereof. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like). A variety of incubation conditions can be used to form the peptide of the present invention. The most preferred conditions are those which mimic physiological conditions.

Production of the stable transfectants, may be accomplished by, for example, transfection of an appropriate cell line with an eukaryotic expression vector, such as pCEP4, in which the coding sequence for the truncated VRP polypeptide or subunit has been cloned into the multiple cloning site. These expression vectors contain a promoter region, such as the human cytomegalovirus promoter (CMV), that drive high-level transcription of desired DNA molecules in a variety of mammalian cells. In addition, these vectors contain genes for the selection of cells that stably express the DNA molecule of interest. The selectable marker in the pCEP4 vector encodes an enzyme that confers resistance to hygromycin, a metabolic inhibitor that is added to the culture to kill the nontransfected cells.

Cells that have stably incorporated the transfected DNA will be identified by their resistance to selection media, as described above, and clonal cell lines will be produced by expansion of resistant colonies. The expression of the truncated VRPs DNA by these cell lines will be assessed by solution hybridization and Northern blot analysis.

Pharmaceutical Compositions and Therapeutic Uses

One object of this invention is to provide truncated VRP in a pharmaceutical composition suitable for therapeutic use. Thus, in one aspect the invention provides a method for stimulating angiogenesis in a patient by administering a therapeutically effective amount of pharmaceutical composition comprising a truncated VRP.

By "therapeutically effective amount" is meant an amount of a compound which produces the desired therapeutic effect in a patient. For example, in reference to a disease or disorder, it is the amount which reduces to some extent one or more symptoms of the disease or disorder, and returns to normal, either partially or completely, physiological or biochemical parameters associated or causative of the disease or disorder. When used to therapeutically treat a patient it is an amount expected to be between 0.1 mg/kg to 100 mg/kg, preferably less than 50 mg/kg, more preferably less than 10 mg/kg, more preferably less than 1 mg/kg. The amount of compound depends on the age, size, and disease associated with the patient.

The optimal formulation and mode of administration of compounds of the present application to a patient depend on factors known in the art such as the particular disease or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry, and sports animals and pets such as horses, dogs and cats.

Preferably, the therapeutically effective amount is provided as a pharmaceutical composition. A pharmacological agent or composition refers to an agent or composition in a form suitable for administration into a multicellular organism such as a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by

injection. Such forms should allow the agent or composition to reach a target cell whether the target cell is present in a multicellular host or in culture. For example, pharmacological agents or compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the agent or composition from exerting its effect.

The claimed compositions can also be formulated as pharmaceutically acceptable salts (*e.g.*, acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfonate, sulfamate, sulfate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclolexylsulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfonic acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclcohexylsulfonic acid, cyclohexylsulfamic acid, and quinic acid. Such salts may be prepared by, for example, reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or

medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, or transmucosally.

The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

The compounds of the invention can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co., Easton, PA, 1990. See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988). A suitable administration format may best be determined by a medical practitioner for each patient individually.

For systemic administration, injection is preferred, *e.g.*, intramuscular, intravenous, intraperitoneal, subcutaneous, intrathecal, or intracerebroventricular. For injection, the

compounds of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. Alternatively, the compounds of the invention are formulated in one or more excipients (*e.g.*, propylene glycol) that are generally accepted as safe as defined by USP standards. They can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 5.6 to 7.4. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example, sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

Systemic administration can also be by transmucosal or transdermal means, or the molecules can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be, for example, through nasal sprays or using suppositories. For oral administration, the molecules are formulated into conventional oral administration dosage forms such as capsules, tablets, and liquid preparations.

For topical administration, the compounds of the invention are formulated into ointments, salves, gels, or creams, as is generally known in the art.

If desired, solutions of the above compositions may be thickened with a thickening agent such as methyl cellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic surfactant (such as a Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

Compositions useful in the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity.

The amounts of various compounds of this invention to be administered can be determined by standard procedures. Generally, a therapeutically effective amount is between about 1 nmole and 3 μmole of the molecule, preferably between about 10 nmole and 1 μmole depending on the age and size of the patient, and the disease or disorder associated with the patient. Generally, it is an amount between about 0.1 and 50 mg/kg, preferably 1 and 20 mg/kg of the animal to be treated.

For use by the physician, the compositions will be provided in dosage unit form containing an amount of a truncated VRP, VRP polypeptide, or VRP subunit.

Gene Therapy

A truncated VRP or its genetic sequences will also be useful in gene therapy (reviewed in Miller, Nature 357:455-460

(1992)). Miller states that advances have resulted in practical approaches to human gene therapy that have demonstrated positive initial results. The basic science of gene therapy is described in Mulligan, Science 260:926-931 (1993). One example of gene therapy is presented in Example 7, which describes the use of adenovirus-mediated gene therapy.

As another example, an expression vector containing the truncated VRP coding sequence may be inserted into cells, the cells are grown *in vitro* and then injected or infused in large numbers into patients. In another example, a DNA segment containing a promoter of choice (for example a strong promoter) is transferred into cells containing an endogenous truncated VRP in such a manner that the promoter segment enhances expression of the endogenous truncated VRP gene (for example, the promoter segment is transferred to the cell such that it becomes directly linked to the endogenous truncated VRP gene).

The gene therapy may involve the use of an adenovirus vector including a nucleotide sequence coding for a truncated VRP subunit, or a naked nucleic acid molecule coding for a truncated VRP subunit. Alternatively, engineered cells containing a nucleic acid molecule coding for a truncated VRP subunit may be injected. Example 7 illustrates a method of gene therapy using an adenovirus vector to provide angiogenesis therapy.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adenovirus, adeno-associated virus, herpes viruses, several RNA viruses, or bovine papilloma virus, may be used for delivery of nucleotide sequences (e.g., cDNA) encoding recombinant truncated VRP subunit into the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing coding sequences. See, for example, the techniques described in Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y.

(1989), and in Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, recombinant nucleic acid molecules encoding protein sequences can be used as naked DNA or in reconstituted system e.g., liposomes or other lipid systems for delivery to target cells (See e.g., Felgner et al., Nature 337:387-8, 1989). Several other methods for the direct transfer of plasmid DNA into cells exist for use in human gene therapy and involve targeting the DNA to receptors on cells by complexing the plasmid DNA to proteins. See, Miller, Nature 357:455-60, 1992.

In its simplest form, gene transfer can be performed by simply injecting minute amounts of DNA into the nucleus of a cell, through a process of microinjection. Capecchi MR, Cell 22:479-88 (1980). Once recombinant genes are introduced into a cell, they can be recognized by the cells normal mechanisms for transcription and translation, and a gene product will be expressed. Other methods have also been attempted for introducing DNA into larger numbers of cells. These methods include: transfection, wherein DNA is precipitated with calcium phosphate and taken into cells by pinocytosis (Chen C. and Okayama H, Mol. Cell Biol. 7:2745-52 (1987)); electroporation, wherein cells are exposed to large voltage pulses to introduce holes into the membrane (Chu G. et al., Nucleic Acids Res., 15:1311-26 (1987)); lipofection/liposome fusion, wherein DNA is packaged into lipophilic vesicles which fuse with a target cell (Felgner PL., et al., Proc. Natl. Acad. Sci. USA. 84:7413-7 (1987)); and particle bombardment using DNA bound to small projectiles (Yang NS. et al., Proc. Natl. Acad. Sci. 87:9568-72 (1990)). Another method for introducing DNA into cells is to couple the DNA to chemically modified proteins.

It has also been shown that adenovirus proteins are capable of destabilizing endosomes and enhancing the uptake of

DNA into cells. The admixture of adenovirus to solutions containing DNA complexes, or the binding of DNA to polylysine covalently attached to adenovirus using protein crosslinking agents substantially improves the uptake and expression of the recombinant gene. Curiel DT et al., Am. J. Respir. Cell. Mol. Biol., 6:247-52 (1992).

In addition, it has been shown that adeno-associated virus vectors may be used for gene delivery into vascular cells (Gnatenko, D., J. of Invest. Med. 45:87-97, (1997)).

As used herein "gene transfer" means the process of introducing a foreign nucleic acid molecule into a cell. Gene transfer is commonly performed to enable the expression of a particular product encoded by the gene. The product may include a protein, polypeptide, anti-sense DNA or RNA, or enzymatically active RNA. Gene transfer can be performed in cultured cells or by direct administration into animals. Generally gene transfer involves the process of nucleic acid molecule contact with a target cell by non-specific or receptor mediated interactions, uptake of nucleic acid molecule into the cell through the membrane or by endocytosis, and release of nucleic acid molecule into the cytoplasm from the plasma membrane or endosome. Expression may require, in addition, movement of the nucleic acid molecule into the nucleus of the cell and binding to appropriate nuclear factors for transcription.

As used herein "gene therapy" is a form of gene transfer and is included within the definition of gene transfer as used herein and specifically refers to gene transfer to express a therapeutic product from a cell *in vivo* or *in vitro*. Gene transfer can be performed ex vivo on cells which are then transplanted into a patient, or can be performed by direct administration of the nucleic acid molecule or nucleic acid-protein complex into the patient.

In another preferred embodiment, a vector having nucleic acid molecule sequences encoding a truncated VRP is provided in which the nucleic acid molecule sequence is expressed only in a specific tissue. Methods of achieving tissue-specific gene expression as set forth in International Publication No. WO 93/09236, filed November 3, 1992 and published May 13, 1993.

In another preferred embodiment, a method of gene replacement is set forth. "Gene replacement" as used herein means supplying a nucleic acid molecule sequence which is capable of being expressed *in vivo* in an animal and thereby providing or augmenting the function of an endogenous gene which is missing or defective in the animal.

In all of the preceding vectors set forth above, a further aspect of the invention is that the nucleic acid sequence contained in the vector may include additions, deletions or modifications to some or all of the sequence of the nucleic acid, as defined above.

Examples

To assist in understanding the present invention, the following Examples are included which describes the results of a series of experiments. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

Example 1

Cloning of N-Terminally Truncated VEGF-B, (des-(1-20)-p21-VEGF-B (or des(2-21)-VEGF-B).

In order to create a novel VEGF-B-related protein that lacks the first 20 amino acids, a cDNA construct is created in the following manner:

A DNA encoding human VEGF-B is amplified from a human heart or skeletal muscle cDNA),or a human fetal brain cDNA library, or a cDNA preparation from another suitable human tissue source by PCR with oligonucleotides corresponding to the published sequence of human VEGF-B. Using standard molecular biology techniques (Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor NY), a DNA fragment then is generated that encodes at its 5' end the signal sequence of human VEGF-B, followed by a codon for proline, the first amino acid residue in mature VEGF-B, and then followed by codons corresponding to amino acids from residues 22 to the C-terminus of human VEGF-B, followed by a stop codon. Appropriate additional non-coding nucleotide sequences are added to the 5' and 3' ends of this DNA construct so as to allow insertion of the DNA into an appropriate expression vector.

In this manner the cleavage site for the signal peptide is preserved in a manner identical to that found in native VEGF-B. However, this strategy results in a change in the new N-terminal amino acid of the truncated VEGF-B. Whereas the normal N-terminal amino acid residue in des(1-20)-VEGF-B is a tyrosine residue:

mspllrrlllvallqlartqa[PVSQFDGPSHQKKVVPWIDV]<u>Y</u>TRAT, the new N-terminal amino acid is proline, and the resulting truncated VEGF-B is equivalent to des(2-21)-VEGF-B):

mspllrrlllvallqlartqaPTRAT...

The change from the native amino acid of the truncated VEGF-B (tyrosine in the case of a a)20-residue truncation) is

not expected to have any effect on the biological activity of the truncated VEGF-B. The advantage of this strategy is that the signal peptide sequence is maintained thus ensuring efficient cleavage of the signal peptide from the precursor during protein processing/secretion.

In another example, truncated VEGF-B, des(1-15)-VEGF-B, is constructed by deleting the first 15 amino acids. The signal peptide cleavage site would be preserved in this case because residue#16 and residue#1 (the new and old N-termini) are identical (proline):

mspllrrillvallqlartqa[PVSQFDGPSHQKKVV]PWIDVYTRAT...

↓

mspllrrillvallqlartqaPWIDVYTRAT..

One of skill in the art would understand that other signal peptides may be used in the present invention. For example, the signal peptide of VEGF-B or VEGF-C could be used which would require that the first amino acid of the truncated protein be an alanine or glycine, respectively, in order to preserve the respective signal peptide cleavage sites. A further alternative would be to use signal peptide sequences from other known proteins; some of these may have cleavage sites compatible with the N-terminal tyrosine of the truncated des(1-20)-VEGF-B.

Another alternative would be to generate a construct that encodes a precursor protein with a cleavage site that incorporates two, rather than one, amino acids from the N-terminus of the original VEGF-B protein sequence. The purpose of this strategy would be to ensure more fully that the cleavage site is compatible with signal peptidase function. This would introduce two new amino acids at the N-terminus of the truncated VEGF-B sequence but such a change would not be expected to alter biological function of the truncated peptide.

The strategy described to generate DNA for expression of des(1-20)-VEGF-B is useful for generation in an analogous manner of VEGF-B mutants with N-terminal truncations of other desired lengths. Further, the strategy is useful to generate N-terminal truncations of other desired lengths in other VEGF-related forms and their isoforms of other species.

Example 2: Expression Of N-Terminally Truncated VEGF-B Subunits

The DNA fragment encoding truncated VEGF-B from Example 1 may be cloned into a suitable plasmid vector.

Sf9 (Sporoptera frugiperda) cells are co-transfected with baculovirus transfer vector pAcUW51 containing cDNA encoding truncated VEGF-B and baculovirus (Baculogold, Pharmingen, San Diego, CA). Selection and plaque purification of recombinant virus are performed according to established protocols using Blue agar overlays (Gibco BRL). High stock of recombinant virus is produced in exponentially growing Sf9 cells using a multiplicity of infection of 0.05. For expression of truncated VEGF-B, Sf9 cells (1x106 cells/ml) growing in serum free medium are infected with recombinant virus at a multiplicity of 10. Supernatant is collected after 72 hours post infection. VEGF expression in baculovirus-infected insect cells, which can be used to express the truncated VRPs of the present invention is also described in Fiebich et al., (Eur. J. Biochem. 211: 19-26, 1993). In this system, VEGF has been shown to be produced in high yield, with efficient glycosylation similar to that seen in mammalian cells. In fact, those skilled in the art will recognize that expression in other systems, including mammalian cell expression systems, is considered to be within the scope of this invention. Methods of expressing VEGF proteins which can be used to express the truncated VRPs of the present invention using baculovirus systems are also provided in references which describe VEGF expression, for example, U.S. Patent Serial No. 5,521,073, and in O'Reilly et al.,

(Baculovirus Expression Vectors: A Laboratory Manual (W.H. Freeman, New York), 1992).

Those skilled in the art will recognize that other expression systems may also be used to express functionally active truncated VRPs.

Functionally active recombinant VEGF isoforms have been expressed in E. Coli (Wilting et al., Dev. Biol. 176, 76-85, 1996) from inclusion body by refolding according to the procedure described previously for homo- and heterodimers of PDGF (Schneppe et al., Gene 143, 201-09, 1994) and in yeast (Mohanraj et al., Biochem. Biophys. Res. Commun. 215:750-56, 1995).

Still other methods of expressing VEGF which can be used to express VRPs in the present invention are described, for example, in Jasny, Science 238:1653, 1987; and Miller et al., In: Genetic Engineering, 1986), Setlow, J.K., et al., eds., Plenum, Vol. 8, pp. 277-297).

Example 3: Purification Of Recombinant Truncated VRPS

For purification of the baculovirus-expressed truncated VEGF-B of Example 2 from insect-cell supernatant, a number of standard techniques can be used. These techniques include, but are not limited to ammonium sulfate precipitation, acetone precipitation, ion-exchange chromatography, size exclusion chromatography, hydrophobic interaction chromatography, reverse-phase HPLC, concanavalin A affinity chromatography, isoelectric focusing, and chromatofocusing. Other standard protein purification techniques are readily obvious to one skilled in the art. For example, proteins with specific tags, such as histidine tags, antigen tags, etc., could be produced by engineering DNA encoding such tags into the VEGF-B DNA such that proteins containing said tags in a manner compatible with the protein's biological activity would be expressed and purified by affinity chromatography directed at the tag. Such

methods are considered within the scope of the present invention.

A preferred purification method for truncated forms of VEGF-B is described in the following: Sf9 Cell supernatant is centrifuged at 10000 rpm for 30 minutes to remove cell debris and viral particles. Supernatant is then concentrated and dialyzed against 20 mM Tris (pH 8.3) for 24 hours. The dialyzed supernatant is centrifuged again to remove insoluble material and loaded onto a Sepharose Q anion exchange column. Protein is eluted from the column by gradient elution using a gradient of NaCl (0 - 1 M NaCl). Chromatography fractions are analyzed by SDS polyacrylamide gel electrophoresis and by ELISA using an antibody that recognizes VEGF-B. Fractions with VEGF-B immunoreactivity are pooled, concentrated, and dialyzed overnight against 0.1% trifluoroacetic acid. Material so prepared is further purified by reverse phase HPLC. Typically approximately 2-5 mg of protein is loaded on a semipreparative C4 column and eluted with a gradient of acetonitrile in 0.1% trifluoracetic acid as described in Esch et al., Meth. Enzymol. 103, 72-89, 1983. Fractions containing truncated VEGF-B are pooled and stored at -80 degrees Celsius until further use.

A preferred method of purification of the basic and heparin-binding N-terminally truncated forms of VEGF-related protein subunits and analogs thereof includes the combined use of heparin-Sepharose affinity chromatography and cation-exchange chromatography, optionally followed by reverse-phase HPLC, essentially as described in Connolly et al., J. Biol. Chem. 264:20017-24, 1989, Gospodarowicz et al., (Proc. Natl. Acad. Sci. USA, 86:7311-15, 1989), or Plouet et al., (Embo J. 8:3801-06, 1989).

Purification is monitored by following the elution of VRP-like material using a number of techniques including radioreceptor assay using ^{125}I-labeled VRP and receptor

preparations consisting of cells or cell membrane preparations in functional assays as described in Examples 4-6.

The truncated VRPs expressed in other eukaryotic cell systems such as yeast or mammalian cells, may be purified in the same manner.

Truncated VRPs expressed in prokaryotic cells will likely need to undergo a re-folding step for proper dimerization of subunits, as described in, for example, Schneppe et al., (Gene 143:201-09, 1994).

Example 4: Receptor-Binding Assay

The binding of truncated VRPs to VEGF receptors can be assessed in various ways. Useful methods include the determination of the ability of VRP analogs to bind to endothelial cells or to cells artificially transfected with KDR, or to soluble forms of the KDR receptor (for example, a KDR/alkaline phosphatase fusion protein (Gitay-Goren et al., J. Biol. Chem. 271:5519-23 (1996)). A preferred procedure has been described by Terman et al. (Biochem. Biophys. Res. Commun. 187:1579-86, 1992).

In this procedure, KDR cDNA is transfected into CMT-3 monkey kidney cells by the DEAE-dextran method by incubating plated cells with DMEM containing 1 μg/ml DNA, 0.5 μg/ml DEAE dextran, and 100 μM chloroquine. Following incubation for 4 hours at 37 degrees Celsius, the medium is aspirated and cells are exposed to 10% DMSO in PBS for one minute. The cells are then washed once with DMEM containing 10% calf serum and then incubated for 40 hours at 37 degrees Celsius in DMEM/10% calf serum containing 100 μM $ZnCl_2$ and 1 μM $CdCl_2$.

VEGF-B is radioiodinated using either the Iodogen method or the chloramine T method. Radiolabelled VEGF-B is separated from excess free iodine-125 using gel filtration on a Sephadez G25 column or a heparin-Sepharose column. Specific activity of radiolabelled ^{125}I-VEGF-B analog should typically be in the

order of 10^5 cpm/ng. For radioceptor assays, CMT-3 (10^5 cells/well) are plated in 12-well plates. Twenty four hours later, cells are washed twice with PBS, and 0.5 ml of DMEM containing 0.15% gelatin and 25 mM HEPES, pH 7.4 is added. ^{125}I-VEGF-B, at concentrations ranging from 1-500 pM, is then added. Binding experiments are done in the presence or absence of 0.5 nM unlabeled VEGF-B for the determination of specific binding. After a 90-minute incubation at room temperature, a 50 µl sample of the media from each well is used to determine the concentration of free radioligand, and the wells are washed 3 times with ice cold PBS containing 0.1% BSA. Cells are extracted from the wells by incubation for 30 minutes with 1% Triton X100 in 100 mM sodium phosphate, pH 8.0, and the radioactivity of the extract is determined in a gamma counter.

Example 5: Mitogenic Assay

The mitogenic activity of truncated VRPs on endothelial cells of human or mammalian origin can be determined by a number of different procedures, including assays where cell proliferation is measured by growth of cell numbers or by incorporation of radioactive DNA precursors (thymidine incorporation) or otherwise appropriately labeled DNA precursors (bromo-deoxyuridine incorporation). These and other methods generally used to determine cell proliferation, including those methods where mitogenic activity is assessed in vivo (for example by determining the mitotix index of endothelial cells) are considered within the scope of this invention. A preferred method is described herein (Bohlen et al., Proc. Natl. Acad. Sci. USA 81:5364-68, 1994): Bovine aortic arch endothelial cells maintained in stock cultures in the presence of Dulbecco's modified Eagle's medium supplemented with 10% calf serum and antibiotics (gentamycin at 50 µg/ml and fungizine at 0.25 µg/ml) and basic fibroblast growth factor (1-10 ng/ml, added every 48 h) are passaged weekly at a split

ratio of 1:64. For mitogenic assays, cell monolayers from stock plates (at passages 3-10) are dissociated using trypsin. Cells are then seeded at a density of approximately 8000 cells/well in 24-well plates in the presence of DMEM and antibiotics as described above. Samples to be assayed (1-10 μl), appropriately diluted in DMEM/0.1% bovine serum albumin), are added six hours after plating of cells and again after 48 hours. After 4 days of culture, endothelial cells are detached from plates with trypsin and counted using a Coulter particle counter.

Another mitogenic activity assay is provided in Olofsson, B. et al., Proc. Natl. Acad. Sci. USA 93:2576-81, 1996). Second passage human umbilical vein endothelial cells (HUVECs) are plated into 96-well plates (4 X 10^3 cells per well) in M-199 medium supplemented with 10% (vol/vol) fetal bovine serum and incubated for 24 hours. Cell culture conditioned medium containing the truncated VRP, in the presence of 1-10 μg/ml heparin, or purified truncated VRP is added to the HUVECs, and the cells are stimulated for 48 hours. Fresh cell culture conditioned medium containing [^{3}H] thymidine (Amersham; 10 μCi/ml) is added to the cells and stimulation is continued for another 48 hours. Cells are washed with PBS and trypsinized and the incorporated radioactivity is determined by liquid scintillation counting. The activity of truncated VRP is compared to the activity of non-truncated VRP.

In another alternative method, bovine capillary endothelial (BCE) cells are seeded into 24-well plates and grown until confluence in minimal essential medium (MEM) supplemented with 10% fetal calf serum. Cells are starved in MEM supplemented with 3% fetal calf serum for 72 hours, after which conditioned medium diluted into serum-free medium is added to the cells and the cells are stimulated for 24 hours. [^{3}H] thymidine is included during the last 4 hours of the stimulation (1 μCi/ml). Cells are washed with PBS and lysed

with NaOH, and incorporated radioactivity is determined by liquid scintillation counting. The activity of truncated VRP is compared to that of non-truncated VRP. Bovine fibroblast growth factor (b-FGF) may be used as an additional control for mitogenic activity, and may also be used to measure its potentiating activity of truncated VRP activity.

Example 6: Angiogenic Activity Of Truncated VRPS

The angiogenic activity of substances can be determined using a variety of in vivo methods. Commonly used methods include the chick chorioallantoic membrane assay, the corneal pouch assay in rabbits, rats, or mice, the matrigel implant assay in mice, the rabbit ear chamber angiogenesis assay, the hamster cheek pouch assay, the Hunt-Schilling chamber model and the rat sponge implant model. Other assay methods to assess the formation of new blood vessels have been described in the literature and are considered to be within the scope of this invention.

A preferred method for demonstrating the angiogenic activity of truncated VRPs is the rabbit corneal pouch assay. In this assay, Elvax (ethylene vinyl acetate) polymer pellets containing approximately 1-1000 ng of the growth factor and a constant amount of rabbit serum albumin as carrier is implanted into a surgical incision in the cornea as described in more detail in Phillips and Knighton, Wound Rep. Reg. 3, 533-539, 1995; Gimbrone et al., J. Natl. Canc. Inst. 52:413-27, 1974; Risau, Proc. Natl. Acad. Sci. USA 83:3855-59, 1986). Growth factor-induced vascularization of the cornea is then observed over a period of 2 weeks. Semiquantitative analysis is possible with morphometric and image analysis techniques using photographs of corneas.

Example 7: Gene-Transfer-Mediated Angiogenesis Therapy Using Truncated VRPS

Truncated VRPs are used for gene-transfer-mediated angiogenesis therapy as described, for example, in PCT/US96/02631, published September 6, 1996 as WO96/26742, hereby incorporated by reference herein in its entirety.

Adenoviral Constructs

A helper independent replication deficient human adenovirus 5 system may be used for gene-transfer. A nucleic acid molecule coding for a truncated VRP subunit may be cloned into the polylinker of plasmid ACCMVPLPA which contains the CMV promoter and SV40 polyadenylation signal flanked by partial adenoviral sequences from which the E1A and E1B genes (essential for viral replication) have been deleted. This plasmid is co-transferred (lipofection) into 293 cells with plasmid JM17 which contains the entire human adenoviral 5 genome with an additional 4.3 kb insert making pJM17 too large to be encapsidated. Homologous rescue recombination results in adenoviral vectors containing the transgene in the absence of E1A/E1B sequences. Although these recombinants are nonreplicative in mammalian cells, they can propagate in 293 cells which have been transformed with E1A/E1B and provided these essential gene products in trans. Transfected cells are monitored for evidence of cytopathic effect which usually occurs 10-14 days after transfection. To identify successful recombinants, cell supernatant from plates showing a cytopathic effect is treated with proteinase K (50 mg/ml with 0.5% sodium dodecyl sulfate and 20 mM EDTA) at 56°C for 60 minutes, phenol/chloroform extracted and ethanol precipitated. Successful recombinants are then identified with PCR using primers (Biotechniques 15:868-72, 1993) complementary to the CMV

promoter and SV40 polyadenylation sequences to amplify the truncated VRP subunit nucleic acid insert and primers (Biotechniques 15:868-72, 1993) designed to concomitantly amplify adenoviral sequences. Successful recombinants then are plaque purified twice. Viral stocks are propagated in 293 cells to titers ranging between 10^{10} and 10^{12} viral particles, and are purified by double CsCl gradient centrifugation prior to use. The system used to generate recombinant adenoviruses imposed a packing limit of 5kb for transgene inserts. The truncated VRP genes, driven by the CMV promoter and with the SV40 polyadenylation sequences are well within the packaging constraints. Recombinant vectors are plaque purified by standard procedures. The resulting viral vectors are propagated on 293 cells to titers in the 10^{10}-10^{12} viral particles range. Cells are infected at 80% confluence and harvested at 36-48 hours. After freeze-thaw cycles the cellular debris is pelleted by standard centrifugation and the virus further purified by double CsCl gradient ultracentrifugation (discontinuous 1.33/1.45 CsCl gradient; cesium prepared in 5 mM Tris, 1 mM EDTA (pH 7.8); 90,000 x g (2 hr), 105,000 x g (18 hr)). Prior to *in vivo* injection, the viral stocks are desalted by gel filtration through Sepharose columns such as G25 Sephadex. The resulting viral stock has a final viral titer approximately in the 10^{10}-10^{12} viral particles range. The adenoviral construct should thus be highly purified, with no wild-type (potentially replicative) virus.

Porcine Ischemia Model For Angiogenesis

A left thoracotomy is performed on domestic pigs (30-40 kg) under sterile conditions for instrumentation. (Hammond, et al. J Clin Invest. 92:2644-52 (1993); Roth, et al. J. Clin. Invest. 91:939-49, 1993). Catheters are placed in the left atrium and aorta, providing a means to measure regional blood flow, and to monitor pressures. Wires are sutured on the left

atrium to permit ECG recording and atrial pacing. Finally, an ameroid constrictor (ameroid), a metal ring including an ameroid substance, is placed around the proximal left circumflex coronary artery (LCx) (Hammond et al. J. Clin. Invest. 92:2644-52 (1993)). After a stable degree of ischemia develops, the treatment group receives an adenoviral construct that includes a truncated VRP gene driven by a CMV promoter. Control animals receive gene transfer with an adenoviral construct that includes a reporter gene, lacZ, driven by a CMV promoter.

Studies are initiated 35 + 3 days after ameroid placement, at a time when collateral vessel development and pacing-induced dysfunction are stable (Roth, et al. Am J Physiol 253:1-11279-1288, 1987, and Roth, et al. Circulation 82:1778-89). Conscious animals are suspended in a sling and pressures from the left ventricle (LV), left atrium (LA) and aorta, and electrocardiogram are recorded in digital format on-line (at rest and during atrial pacing at 200 bpm). Two-dimensional and M-mode images are obtained using a Hewlett Packard ultrasound imaging system. Images are obtained from a right parasternal approach at the mid-papillary muscle level and recorded on VHS tape. Images are recorded with animals in a basal state and again during right atrial pacing (HR=200 bpm). These studies are performed one day prior to gene transfer and repeated 14 + 1 days later. Rate-pressure products and left atrial pressures should be similar in both groups before and after gene transfer, indicating similar myocardial oxygen demands and loading conditions. Echocardiographic measurements are made using standardized criteria (Sahn, et al. *Circulation* 58:1072, 1978). End-diastolic wall thickness (EDWTh) and end-systolic wall thickness (ESWTh) are measured from 5 continuous beats and averaged. Percent wall thickening (%WTh) is calculated [(EDWTh-ESWTh)/EDWTh] X 100. Data should be analyzed without knowledge of which gene the animals had received. To

demonstrate reproducibility of echocardiographic measurements, animals should be imaged on two consecutive days, showing high correlation (r^2=0.90; p=0.005).

35 ± 3 days after ameroid placement, well after ameroid closure, but before gene transfer, contrast echocardiographic studies are performed using the contrast material (Levovist) which is injected into the left atrium during atrial pacing (200 bprn). Studies are repeated 14 ± 1 days after gene transfer. Peak contrast intensity is measured from the video images using a computer-based video analysis program (Color Vue II, Nova Microsonics, Indianapolis, Indiana), that provides an objective measure of video intensity. The contrast studies are analyzed without knowledge of which gene the animals have received.

At completion of the study, animals are anesthetized and midline thoracotomy performed. The brachycephalic artery is isolated, a canula inserted, and other great vessels ligated. The animals receive intravenous heparin (10,000 IU) and papaverine (60 mg). Potassium chloride is given to induce diastolic cardiac arrest, and the aorta cross-clamped. Saline is delivered through the brachycephalic artery cannula (120 mmHg pressure), thereby perfusing the coronary arteries. Glutaraldehyde solution (6.25%, 0.1 M cacodylate buffer) was perfused (120 mmH pressure) until the heart is well fixed (10-15 min). The heart is then removed, the beds identified using color-coded dyes injected anterograde through the left anterior descending (LAD), left circumflex (LCx), and right coronary arteries. The ameroid is examined to confirm closure. Samples taken from the normally perfused and ischemic regions are divided into thirds and the endocardial and epicardial thirds are plastic-imbedded. Microscopic analysis to quantitate capillary number is conducted as previously described (Mathieu-Costello, et al. Am J Physiol 359:H204, 1990). Four 1 μm thick transverse sections are taken from each subsample (endocardium

and epicardium of each region) and point-counting is used to determine capillary number per fiber number ratio at 400X magnification. Twenty to twenty-five high power fields are counted per subsample. Within each region, capillary number to fiber number rations should be similar in endocardium and epicardium so the 40-50 field per region should be averaged to provide the transmural capillary to fiber number ratio.

To establish that improved regional function and blood flow result from transgene expression, PCR and RT-PCR may be used to detect transgenic truncated VRP DNA and mRNA in myocardium from animals that have received truncated VRP gene transfer. Using a sense primer to the CMV promoter [GCAGAGCTCGTTTAGTGAAC] [SEQ I.D. NO. 41]; and an antisense primer to the internal truncated VRP subunit sequence, PCR is used to amplify the expected 500 bp fragment. Using a sense primer to the beginning of the truncated VRP subunit sequence, and an antisense primer to the internal truncated VRP sequence, RT-PCR is used to amplify the expected 400 bp fragment.

Finally, using a polyclonal antibody directed against VRP, truncated VRP expression may be demonstrated 48 hours as well as 14 ± 1 days after gene transfer in cells and myocardium from animals that have received gene transfer with a truncated VRP gene.

The helper independent replication deficient human adenovirus 5 system is used to prepare transgene containing vectors. The material injected in vivo should be highly purified and contain no wild-type (replication competent) adenovirus. Thus adenoviral infection and inflammatory infiltration in the heart are minimized. By injecting the material directly into the lumen of the coronary artery by coronary catheters, it is possible to target the gene effectively. When delivered in this manner there should be no transgene expression in hepatocytes, and viral RNA should not

be found in the urine at any time after intracoronary injection.

Injection of the construct (4.0 ml containing about 10^{11} viral particles of adenovirus) is performed by injecting 2.0 ml into both the left and right coronary arteries (collateral flow to the LCx bed appeared to come from both vessels). Animals are anesthetized, and arterial access acquired via the right carotid by cut-down; a 5F Cordis sheath is then placed. A 5F Multipurpose (A2) coronary catheter is used to engage the coronary arteries. Closure of the LCx ameroid is confirmed by contrast injection into the left main coronary artery. The catheter tip is then placed 1 cm within the arterial lumen so that minimal material is lost to the proximal aorta during injection. This procedure is carried out for each of the pigs.

Once gene transfer is performed, three strategies are used to establish successful incorporation and expression of the gene. (1) Some constructs may include a reporter gene (lacZ); (2) myocardium from the relevant beds is sampled, and immunoblotting is performed to quantitate the presence of truncated VRP and (3) PCR is used to detect truncated VRP mRNA and DNA.

The regional contractile function data obtained should show that control pigs show a similar degree of pacing-induced dysfunction in the ischemic region before and 14 $\pm$ 1 days after gene transfer. In contrast, pigs receiving truncated gene transfer should show an increase in wall thickening in the ischemic region during pacing, demonstrating that truncated VRP subunit gene transfer in accordance with the invention is associated with improved contraction in the ischemic region during pacing. Wall thickening in the normally perfused region (the interventricular septum) should be normal during pacing and unaffected by gene transfer. The percent decrease in function measured by transthoracic echocardiography should be very similar to the percentage decrease measured by

sonomicrometry during atrial pacing in the same model (Hammond, et al. J. Clin. Invest. 92:2644, 1993), documenting the accuracy of echocardiography for the evaluation of ischemic dysfunction.

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANT: Collateral Therapeutics

(ii) TITLE OF INVENTION: TRUNCATED VEGF-RELATED PROTEINS

(iii) NUMBER OF SEQUENCES: 41

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET: 633 West Fifth Street Suite 4700
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: U.S.A.
(F) ZIP: 90071-2066

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: IBM P.C. DOS 5.0
(D) SOFTWARE: FastSEQ for Windows 2.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 08/842,984
(B) FILING DATE: April 25, 1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Warburg, Richard J.
(B) REGISTRATION NUMBER: 32,327
(C) REFERENCE/DOCKET NUMBER: 221/062

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (213) 489-1600
(B) TELEFAX: (213) 955-0440
(C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 188 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Val Ala Leu Leu Gln Leu
1               5                   10                  15

Ala Arg Thr Gln Ala Pro Val Ser Gln Phe Asp Gly Pro Ser His Gln
            20                  25                  30

Lys Lys Val Val Pro Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Ser Met Glu Leu Met Gly Asn Val
    50                  55                  60

Val Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Gln Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Glu Ser Ala Val Lys Pro Asp Ser Pro Arg Ile Leu Cys Pro Pro
    130                 135                 140

Cys Thr Gln Arg Arg Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
145                 150                 155                 160

Cys Arg Arg Arg Arg Phe Leu His Cys Gln Gly Arg Gly Leu Glu Leu
                165                 170                 175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Pro Arg Lys
            180                 185
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1               5                   10                  15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30
```

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65 70 75 80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
85 90 95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
100 105 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
115 120 125

Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg Pro
130 135 140

Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser
145 150 155 160

Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala His
165 170 175

Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala Ala
180 185 190

Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
195 200 205

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 419 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1 5 10 15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
100 105 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
115 120 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
130 135 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
145 150 155 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
165 170 175

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
180 185 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
195 200 205

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
210 215 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225 230 235 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
245 250 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
260 265 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
275 280 285

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
290 295 300

Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
305 310 315 320

Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385 390 395 400

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Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
                405                 410                 415

Gln Met Ser
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
 1               5                  10                  15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
            20                  25                  30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
        35                  40                  45

Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Val Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Ser Gln His Ile Gly Glu Met Ser Phe Leu Gln His Ser Lys Cys
        115                 120                 125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
    130                 135                 140

Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys
145                 150                 155                 160

His Leu Cys Gly Asp Ala Val Pro Arg Arg
                165                 170
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 221 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Arg Arg Cys Arg Ile Ser Gly Arg Pro Pro Ala Pro Pro Gly Val
1 5 10 15

Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln Arg
20 25 30

Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro
35 40 45

Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala
50 55 60

Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys
65 70 75 80

Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val
85 90 95

Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu
100 105 110

Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys
115 120 125

Asp Ser Ala Val Lys Gln Asp Arg Ala Ala Thr Pro His His Arg Pro
130 135 140

Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser
145 150 155 160

Pro Ala Asp Ile Thr Gln Ser His Ser Ser Pro Arg Pro Leu Cys Pro
165 170 175

Arg Cys Thr Gln His His Gln Cys Pro Asp Pro Arg Thr Cys Arg Cys
180 185 190

Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu
195 200 205

Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
210 215 220

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Lys Leu Leu Val Gly Ile Leu Val Ala Val Cys Leu His Gln Tyr
1 5 10 15

Leu Leu Asn Ala Asp Ser Asn Thr Lys Gly Trp Ser Glu Val Leu Lys
20 25 30

Gly Ser Glu Cys Lys Pro Arg Pro Ile Val Val Pro Val Ser Glu Thr
35 40 45

His Pro Glu Leu Thr Ser Gln Arg Phe Asn Pro Pro Cys Val Thr Leu
50 55 60

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser Leu Glu Cys Val Pro
65 70 75 80

Thr Glu Glu Val Asn Val Thr Met Glu Leu Leu Gly Ala Ser Gly Ser
85 90 95

Gly Ser Asn Gly Met Gln Arg Leu Ser Phe Val Glu His Lys Lys Cys
100 105 110

Asp Cys Arg Pro Arg Phe Thr Thr Thr Pro Pro Thr Thr Thr Arg Pro
115 120 125

Pro Arg Arg Arg Arg
130

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 148 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Lys Leu Thr Ala Thr Leu Gln Val Val Val Ala Leu Leu Ile Cys
1 5 10 15

Met Tyr Asn Leu Pro Glu Cys Val Ser Gln Ser Asn Asp Ser Pro Pro
20 25 30

Ser Thr Asn Asp Trp Met Arg Thr Leu Asp Lys Ser Gly Cys Lys Pro
35 40 45

Arg Asp Thr Val Val Tyr Leu Gly Glu Glu Tyr Pro Glu Ser Thr Asn
50 55 60

Leu Gln Tyr Asn Pro Arg Cys Val Thr Val Lys Arg Cys Ser Gly Cys
65 70 75 80

Cys Asn Gly Asp Gly Gln Ile Cys Thr Ala Val Glu Thr Arg Asn Thr
85 90 95

Thr Val Thr Val Ser Val Thr Gly Val Ser Ser Ser Ser Gly Thr Asn
100 105 110

Ser Gly Val Ser Thr Asn Leu Gln Arg Ile Ser Val Thr Glu His Thr
115 120 125

Lys Cys Asp Cys Ile Gly Arg Thr Thr Thr Thr Pro Thr Thr Thr Arg

130 135 140

Glu Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 160 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Pro Ser His Gln Lys Lys Val Val Pro Trp Ile Asp Val Tyr Thr Arg
 1               5                  10                  15

Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Ser Met Glu Leu
            20                  25                  30

Met Gly Asn Val Val Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln
        35                  40                  45

Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr
    50                  55                  60

Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Gln Tyr Pro Ser
65                  70                  75                  80

Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys
                85                  90                  95

Arg Pro Lys Lys Lys Glu Ser Ala Val Lys Pro Asp Ser Pro Arg Ile
            100                 105                 110

Leu Cys Pro Pro Cys Thr Gln Arg Arg Gln Arg Pro Asp Pro Arg Thr
        115                 120                 125

Cys Arg Cys Arg Cys Arg Arg Arg Arg Phe Leu His Cys Gln Gly Arg
    130                 135                 140

Gly Leu Glu Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Pro Arg Lys
145                 150                 155                 160
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Lys Val Val Pro Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro
1               5                   10                  15

Arg Glu Val Val Val Pro Leu Ser Met Glu Leu Met Gly Asn Val Val
            20                  25                  30

Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys
        35                  40                  45

Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val
    50                  55                  60

Arg Met Gln Ile Leu Met Ile Gln Tyr Pro Ser Ser Gln Leu Gly Glu
65                  70                  75                  80

Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys
                85                  90                  95

Glu Ser Ala Val Lys Pro Asp Ser Pro Arg Ile Leu Cys Pro Pro Cys
            100                 105                 110

Thr Gln Arg Arg Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys
        115                 120                 125

Arg Arg Arg Arg Phe Leu His Cys Gln Gly Arg Gly Leu Glu Leu Asn
    130                 135                 140

Pro Asp Thr Cys Arg Cys Arg Lys Pro Arg Lys
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 152 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Pro Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val
1               5                   10                  15

Val Val Pro Leu Ser Met Glu Leu Met Gly Asn Val Val Lys Gln Leu
            20                  25                  30

Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp
        35                  40                  45

Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln
    50                  55                  60

Ile Leu Met Ile Gln Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu
65                  70                  75                  80

Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Glu Ser Ala
                85                  90                  95

Val Lys Pro Asp Ser Pro Arg Ile Leu Cys Pro Pro Cys Thr Gln Arg
```

```
                100                 105                 110

Arg Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg
        115                 120                 125

Arg Phe Leu His Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr
    130                 135                 140

Cys Arg Cys Arg Lys Pro Arg Lys
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val
1               5                   10                  15

Pro Leu Ser Met Glu Leu Met Gly Asn Val Val Lys Gln Leu Val Pro
            20                  25                  30

Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly
        35                  40                  45

Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile Leu
    50                  55                  60

Met Ile Gln Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu
65                  70                  75                  80

His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Glu Ser Ala Val Lys
                85                  90                  95

Pro Asp Ser Pro Arg Ile Leu Cys Pro Pro Cys Thr Gln Arg Arg Gln
            100                 105                 110

Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Arg Phe
        115                 120                 125

Leu His Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys Arg
    130                 135                 140

Cys Arg Lys Pro Arg Lys
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Ser
1 5 10 15

Met Glu Leu Met Gly Asn Val Val Lys Gln Leu Val Pro Ser Cys Val
20 25 30

Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys
35 40 45

Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Gln
50 55 60

Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln
65 70 75 80

Cys Glu Cys Arg Pro Lys Lys Lys Glu Ser Ala Val Lys Pro Asp Ser
85 90 95

Pro Arg Ile Leu Cys Pro Pro Cys Thr Gln Arg Arg Gln Arg Pro Asp
100 105 110

Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Arg Phe Leu His Cys
115 120 125

Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys
130 135 140

Pro Arg Lys
145

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 145 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Ser Met Glu
1 5 10 15

Leu Met Gly Asn Val Val Lys Gln Leu Val Pro Ser Cys Val Thr Val
20 25 30

Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro
35 40 45

Thr Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Gln Tyr Pro
50 55 60

Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu
65 70 75 80

```
Cys Arg Pro Lys Lys Lys Glu Ser Ala Val Lys Pro Asp Ser Pro Arg
                85                  90                  95

Ile Leu Cys Pro Pro Cys Thr Gln Arg Arg Gln Arg Pro Asp Pro Arg
            100                 105                 110

Thr Cys Arg Cys Arg Cys Arg Arg Arg Arg Phe Leu His Cys Gln Gly
        115                 120                 125

Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Pro Arg
    130                 135                 140

Lys
145
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 178 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Pro Gly His Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg
 1               5                  10                  15

Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu
            20                  25                  30

Met Gly Thr Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln
        35                  40                  45

Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr
    50                  55                  60

Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser
65                  70                  75                  80

Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys
                85                  90                  95

Arg Pro Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro
            100                 105                 110

His His Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro
        115                 120                 125

Gly Ala Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly
    130                 135                 140

Pro Ser Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly
145                 150                 155                 160

Pro Ala Ala Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly
                165                 170                 175

Gly Ala
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro
1               5                   10                  15

Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala
            20                  25                  30

Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys
        35                  40                  45

Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val
    50                  55                  60

Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu
65                  70                  75                  80

Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Asp
                85                  90                  95

Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg Pro Gln
            100                 105                 110

Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser Pro
        115                 120                 125

Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala His Ala
    130                 135                 140

Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala Ala Ala
145                 150                 155                 160

Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
                165                 170
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 168 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val
1               5                   10                  15

Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala Lys Gln Leu Val Pro
            20                  25                  30
```

```
Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly
        35                  40                  45

Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile Leu
    50                  55                  60

Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu
65                  70                  75                  80

His Ser Gln Cys Glu Cys Arg Pro Lys Lys Asp Ser Ala Val Lys Pro
                85                  90                  95

Asp Arg Ala Ala Thr Pro His His Arg Pro Gln Pro Arg Ser Val Pro
            100                 105                 110

Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser Pro Ala Asp Ile Thr His
        115                 120                 125

Pro Thr Pro Ala Pro Gly Pro Ser Ala His Ala Ala Pro Ser Thr Thr
    130                 135                 140

Ser Ala Leu Thr Pro Gly Pro Ala Ala Ala Ala Ala Asp Ala Ala Ala
145                 150                 155                 160

Ser Ser Val Ala Lys Gly Gly Ala
                165
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 163 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu
 1               5                  10                  15

Leu Met Gly Thr Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val
            20                  25                  30

Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro
        35                  40                  45

Thr Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro
    50                  55                  60

Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu
65                  70                  75                  80

Cys Arg Pro Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr
                85                  90                  95

Pro His His Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala
            100                 105                 110

Pro Gly Ala Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro
```

115 120 125

Gly Pro Ser Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro
130 135 140

Gly Pro Ala Ala Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys
145 150 155 160

Gly Gly Ala

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Pro Gly His Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg
1 5 10 15

Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu
20 25 30

Met Gly Thr Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln
35 40 45

Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr
50 55 60

Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser
65 70 75 80

Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys
85 90 95

Arg Pro Lys Lys Lys Asp Ser Ala Val Lys Gln Asp Arg Ala Ala Thr
100 105 110

Pro His His Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala
115 120 125

Pro Gly Ala Pro Ser Pro Ala Asp Ile Thr Gln Ser His Ser Ser Pro
130 135 140

Arg Pro Leu Cys Pro Arg Cys Thr Gln His His Gln Cys Pro Asp Pro
145 150 155 160

Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln
165 170 175

Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu
180 185 190

Arg Arg

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 189 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro
 1               5                  10                  15

Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala
            20                  25                  30

Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys
        35                  40                  45

Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val
    50                  55                  60

Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu
65                  70                  75                  80

Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys
                85                  90                  95

Asp Ser Ala Val Lys Gln Asp Arg Ala Ala Thr Pro His His Arg Pro
            100                 105                 110

Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser
        115                 120                 125

Pro Ala Asp Ile Thr Gln Ser His Ser Ser Pro Arg Pro Leu Cys Pro
    130                 135                 140

Arg Cys Thr Gln His His Gln Cys Pro Asp Pro Arg Thr Cys Arg Cys
145                 150                 155                 160

Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu
                165                 170                 175

Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
            180                 185
```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 184 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val
 1               5                  10                  15

Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala Lys Gln Leu Val Pro
```

```
                20                      25                      30
Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly
            35                      40                      45

Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile Leu
        50                      55                      60

Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu
65                      70                      75                      80

His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Asp Ser Ala Val Lys
                85                      90                      95

Gln Asp Arg Ala Ala Thr Pro His His Arg Pro Gln Pro Arg Ser Val
            100                     105                     110

Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser Pro Ala Asp Ile Thr
        115                     120                     125

Gln Ser His Ser Ser Pro Arg Pro Leu Cys Pro Arg Cys Thr Gln His
    130                     135                     140

His Gln Cys Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg
145                     150                     155                     160

Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr
                165                     170                     175

Cys Arg Cys Arg Lys Leu Arg Arg
            180
```

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 179 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
Arg Ala Thr Cys Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu
1               5                   10                  15

Leu Met Gly Thr Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val
            20                  25                  30

Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro
        35                  40                  45

Thr Gly Gln His Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro
    50                  55                  60

Ser Ser Gln Leu Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu
65                  70                  75                  80

Cys Arg Pro Lys Lys Lys Asp Ser Ala Val Lys Gln Asp Arg Ala Ala
                85                  90                  95
```

Thr Pro His His Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser
100 105 110

Ala Pro Gly Ala Pro Ser Pro Ala Asp Ile Thr Gln Ser His Ser Ser
115 120 125

Pro Arg Pro Leu Cys Pro Arg Cys Thr Gln His His Gln Cys Pro Asp
130 135 140

Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys
145 150 155 160

Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys
165 170 175

Leu Arg Arg

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 307 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
1 5 10 15

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
20 25 30

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
35 40 45

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
50 55 60

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
65 70 75 80

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
85 90 95

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
100 105 110

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
115 120 125

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
130 135 140

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
145 150 155 160

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
165 170 175

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
180 185 190

Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
195 200 205

Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
210 215 220

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
225 230 235 240

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
245 250 255

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
260 265 270

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
275 280 285

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
290 295 300

Gln Met Ser
305

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 302 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro
1 5 10 15

Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn
20 25 30

Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
35 40 45

Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
50 55 60

Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
65 70 75 80

Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
85 90 95

Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
100 105 110

Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
115 120 125

Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
130 135 140

Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
145 150 155 160

Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
165 170 175

Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
180 185 190

Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
195 200 205

Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
210 215 220

Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
225 230 235 240

Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
245 250 255

Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
260 265 270

Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
275 280 285

Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
290 295 300

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile
1 5 10 15

Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro
20 25 30

Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly
35 40 45

Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe
50 55 60

Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser
65 70 75 80

```
Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr
                85                  90                  95

Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro
            100                 105                 110

Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn
        115                 120                 125

Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser
    130                 135                 140

Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro
145                 150                 155                 160

Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly
                165                 170                 175

Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser
            180                 185                 190

Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala
        195                 200                 205

Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr
    210                 215                 220

Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys
225                 230                 235                 240

Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His
                245                 250                 255

Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala
            260                 265                 270

Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro
        275                 280                 285

Ser Tyr Trp Lys Arg Pro Gln Met Ser
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 292 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu
1               5                   10                  15

Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val
            20                  25                  30

Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn
```

```
        35                  40                  45
Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro
    50                  55                  60
Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr
65                  70                  75                  80
Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser
                85                  90                  95
Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala
            100                 105                 110
Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg
        115                 120                 125
Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp
    130                 135                 140
Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp
145                 150                 155                 160
Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser
                165                 170                 175
Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys
            180                 185                 190
Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp
        195                 200                 205
Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln
    210                 215                 220
Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln
225                 230                 235                 240
Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys
                245                 250                 255
Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe
            260                 265                 270
Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg
        275                 280                 285
Pro Gln Met Ser
    290
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Leu Asn Ala Asp Ser Asn Thr Lys Gly Trp Ser Glu Val Leu Lys Gly
1 5 10 15

Ser Glu Cys Lys Pro Arg Pro Ile Val Val Pro Val Ser Glu Thr His
20 25 30

Pro Glu Leu Thr Ser Gln Arg Phe Asn Pro Pro Cys Val Thr Leu Met
35 40 45

Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser Leu Glu Cys Val Pro Thr
50 55 60

Glu Glu Val Asn Val Thr Met Glu Leu Leu Gly Ala Ser Gly Ser Gly
65 70 75 80

Ser Asn Gly Met Gln Arg Leu Ser Phe Val Glu His Lys Lys Cys Asp
85 90 95

Cys Arg Pro Arg Phe Thr Thr Thr Pro Pro Thr Thr Thr Arg Pro Pro
100 105 110

Arg Arg Arg Arg
115

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Thr Lys Gly Trp Ser Glu Val Leu Lys Gly Ser Glu Cys Lys Pro
1 5 10 15

Arg Pro Ile Val Val Pro Val Ser Glu Thr His Pro Glu Leu Thr Ser
20 25 30

Gln Arg Phe Asn Pro Pro Cys Val Thr Leu Met Arg Cys Gly Gly Cys
35 40 45

Cys Asn Asp Glu Ser Leu Glu Cys Val Pro Thr Glu Glu Val Asn Val
50 55 60

Thr Met Glu Leu Leu Gly Ala Ser Gly Ser Gly Ser Asn Gly Met Gln
65 70 75 80

Arg Leu Ser Phe Val Glu His Lys Lys Cys Asp Cys Arg Pro Arg Phe
85 90 95

Thr Thr Thr Pro Pro Thr Thr Thr Arg Pro Pro Arg Arg Arg Arg
100 105 110

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

```
Ser Glu Val Leu Lys Gly Ser Glu Cys Lys Pro Arg Pro Ile Val Val
 1               5                  10                  15

Pro Val Ser Glu Thr His Pro Glu Leu Thr Ser Gln Arg Phe Asn Pro
            20                  25                  30

Pro Cys Val Thr Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser
        35                  40                  45

Leu Glu Cys Val Pro Thr Glu Glu Val Asn Val Thr Met Glu Leu Leu
    50                  55                  60

Gly Ala Ser Gly Ser Gly Ser Asn Gly Met Gln Arg Leu Ser Phe Val
65                  70                  75                  80

Glu His Lys Lys Cys Asp Cys Arg Pro Arg Phe Thr Thr Thr Pro Pro
                85                  90                  95

Thr Thr Thr Arg Pro Pro Arg Arg Arg Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```
Gly Ser Glu Cys Lys Pro Arg Pro Ile Val Val Pro Val Ser Glu Thr
 1               5                  10                  15

His Pro Glu Leu Thr Ser Gln Arg Phe Asn Pro Pro Cys Val Thr Leu
            20                  25                  30

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser Leu Glu Cys Val Pro
        35                  40                  45

Thr Glu Glu Val Asn Val Thr Met Glu Leu Leu Gly Ala Ser Gly Ser
    50                  55                  60

Gly Ser Asn Gly Met Gln Arg Leu Ser Phe Val Glu His Lys Lys Cys
65                  70                  75                  80

Asp Cys Arg Pro Arg Phe Thr Thr Thr Pro Pro Thr Thr Thr Arg Pro
                85                  90                  95

Pro Arg Arg Arg Arg
            100
```

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
Asn Asp Ser Pro Pro Ser Thr Asn Asp Trp Met Arg Thr Leu Asp Lys
 1               5                  10                  15

Ser Gly Cys Lys Pro Arg Asp Thr Val Val Tyr Leu Gly Glu Glu Tyr
            20                  25                  30

Pro Glu Ser Thr Asn Leu Gln Tyr Asn Pro Arg Cys Val Thr Val Lys
        35                  40                  45

Arg Cys Ser Gly Cys Cys Asn Gly Asp Gly Gln Ile Cys Thr Ala Val
    50                  55                  60

Glu Thr Arg Asn Thr Thr Val Thr Val Ser Val Thr Gly Val Ser Ser
65                  70                  75                  80

Ser Ser Gly Thr Asn Ser Gly Val Ser Thr Asn Leu Gln Arg Ile Ser
                85                  90                  95

Val Thr Glu His Thr Lys Cys Asp Cys Ile Gly Arg Thr Thr Thr Thr
            100                 105                 110

Pro Thr Thr Thr Arg Glu Pro Arg Arg
        115                 120
```

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
Ser Thr Asn Asp Trp Met Arg Thr Leu Asp Lys Ser Gly Cys Lys Pro
 1               5                  10                  15

Arg Asp Thr Val Val Tyr Leu Gly Glu Glu Tyr Pro Glu Ser Thr Asn
            20                  25                  30

Leu Gln Tyr Asn Pro Arg Cys Val Thr Val Lys Arg Cys Ser Gly Cys
        35                  40                  45

Cys Asn Gly Asp Gly Gln Ile Cys Thr Ala Val Glu Thr Arg Asn Thr
    50                  55                  60

Thr Val Thr Val Ser Val Thr Gly Val Ser Ser Ser Ser Gly Thr Asn
```

65 70 75 80

Ser Gly Val Ser Thr Asn Leu Gln Arg Ile Ser Val Thr Glu His Thr
85 90 95

Lys Cys Asp Cys Ile Gly Arg Thr Thr Thr Thr Pro Thr Thr Thr Arg
100 105 110

Glu Pro Arg Arg
115

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Met Arg Thr Leu Asp Lys Ser Gly Cys Lys Pro Arg Asp Thr Val Val
1 5 10 15

Tyr Leu Gly Glu Glu Tyr Pro Glu Ser Thr Asn Leu Gln Tyr Asn Pro
20 25 30

Arg Cys Val Thr Val Lys Arg Cys Ser Gly Cys Cys Asn Gly Asp Gly
35 40 45

Gln Ile Cys Thr Ala Val Glu Thr Arg Asn Thr Thr Val Thr Val Ser
50 55 60

Val Thr Gly Val Ser Ser Ser Ser Gly Thr Asn Ser Gly Val Ser Thr
65 70 75 80

Asn Leu Gln Arg Ile Ser Val Thr Glu His Thr Lys Cys Asp Cys Ile
85 90 95

Gly Arg Thr Thr Thr Thr Pro Thr Thr Thr Arg Glu Pro Arg Arg
100 105 110

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Lys Ser Gly Cys Lys Pro Arg Asp Thr Val Val Tyr Leu Gly Glu Glu
1 5 10 15

Tyr Pro Glu Ser Thr Asn Leu Gln Tyr Asn Pro Arg Cys Val Thr Val
20 25 30

```
Lys Arg Cys Ser Gly Cys Cys Asn Gly Asp Gly Gln Ile Cys Thr Ala
        35                  40                  45

Val Glu Thr Arg Asn Thr Thr Val Thr Val Ser Val Thr Gly Val Ser
    50                  55                  60

Ser Ser Ser Gly Thr Asn Ser Gly Val Ser Thr Asn Leu Gln Arg Ile
65                  70                  75                  80

Ser Val Thr Glu His Thr Lys Cys Asp Cys Ile Gly Arg Thr Thr Thr
                85                  90                  95

Thr Pro Thr Thr Thr Arg Glu Pro Arg Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 167 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

```
Pro Val Ser Gln Phe Asp Gly Pro Ser His Gln Lys Lys Val Val Pro
1               5                   10                  15

Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val
            20                  25                  30

Val Pro Leu Ser Met Glu Leu Met Gly Asn Val Val Lys Gln Leu Val
        35                  40                  45

Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp
    50                  55                  60

Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile
65                  70                  75                  80

Leu Met Ile Gln Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu
                85                  90                  95

Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Glu Ser Ala Val
            100                 105                 110

Lys Pro Asp Ser Pro Arg Ile Leu Cys Pro Pro Cys Thr Gln Arg Arg
        115                 120                 125

Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Arg
    130                 135                 140

Phe Leu His Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys
145                 150                 155                 160

Arg Cys Arg Lys Pro Arg Lys
                165
```

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 185 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```
Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln Arg Lys Val Val Ser
1               5                   10                  15

Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val
            20                  25                  30

Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala Lys Gln Leu Val
        35                  40                  45

Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp
    50                  55                  60

Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile
65                  70                  75                  80

Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu
                85                  90                  95

Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Asp Ser Ala Val Lys
            100                 105                 110

Pro Asp Arg Ala Ala Thr Pro His His Arg Pro Gln Pro Arg Ser Val
        115                 120                 125

Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser Pro Ala Asp Ile Thr
    130                 135                 140

His Pro Thr Pro Ala Pro Gly Pro Ser Ala His Ala Ala Pro Ser Thr
145                 150                 155                 160

Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala Ala Ala Ala Asp Ala Ala
                165                 170                 175

Ala Ser Ser Val Ala Lys Gly Gly Ala
            180                 185
```

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 201 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```
Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln Arg Lys Val Val Ser
1               5                   10                  15
```

```
Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val
            20                  25                  30

Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala Lys Gln Leu Val
        35                  40                  45

Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp
    50                  55                  60

Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile
65                  70                  75                  80

Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu
                85                  90                  95

Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Asp Ser Ala Val
            100                 105                 110

Lys Gln Asp Arg Ala Ala Thr Pro His His Arg Pro Gln Pro Arg Ser
        115                 120                 125

Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser Pro Ala Asp Ile
    130                 135                 140

Thr Gln Ser His Ser Ser Pro Arg Pro Leu Cys Pro Arg Cys Thr Gln
145                 150                 155                 160

His His Gln Cys Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg
                165                 170                 175

Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp
            180                 185                 190

Thr Cys Arg Cys Arg Lys Leu Arg Arg
        195                 200
```

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

```
Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser Gly Leu
1               5                   10                  15

Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala Tyr Ala
            20                  25                  30

Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu
        35                  40                  45

Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln
    50                  55                  60
```

```
Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn
65                  70                  75                  80

Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr
                85                  90                  95

Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met
            100                 105                 110

Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr
        115                 120                 125

Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly
    130                 135                 140

Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr
145                 150                 155                 160

Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro
                165                 170                 175

Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met
            180                 185                 190

Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser
        195                 200                 205

Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro
    210                 215                 220

Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu
225                 230                 235                 240

Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe
                245                 250                 255

His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln
            260                 265                 270

Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys
        275                 280                 285

Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe
    290                 295                 300

Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln
305                 310                 315                 320

Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly
                325                 330                 335

Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys
            340                 345                 350

Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys
        355                 360                 365

Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu
    370                 375                 380

Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
385                 390                 395
```

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

```
Met Lys Leu Leu Val Gly Ile Leu Val Ala Val Cys Leu His Gln Tyr
 1               5                  10                  15

Leu Leu Asn Ala Asp Ser Asn Thr Lys Gly Trp Ser Glu Val Leu Lys
            20                  25                  30

Gly Ser Glu Cys Lys Pro Arg Pro Ile Val Val Pro Val Ser Glu Thr
        35                  40                  45

His Pro Glu Leu Thr Ser Gln Arg Phe Asn Pro Pro Cys Val Thr Leu
    50                  55                  60

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser Leu Glu Cys Val Pro
65                  70                  75                  80

Thr Glu Glu Val Asn Val Thr Met Glu Leu Leu Gly Ala Ser Gly Ser
                85                  90                  95

Gly Ser Asn Gly Met Gln Arg Leu Ser Phe Val Glu His Lys Lys Cys
            100                 105                 110

Asp Cys Arg Pro Arg Phe Thr Thr Thr Pro Pro Thr Thr Thr Arg Pro
        115                 120                 125

Pro Arg Arg Arg Arg
    130
```

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 148 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```
Met Lys Leu Thr Ala Thr Leu Gln Val Val Val Ala Leu Leu Ile Cys
 1               5                  10                  15

Met Tyr Asn Leu Pro Glu Cys Val Ser Gln Ser Asn Asp Ser Pro Pro
            20                  25                  30

Ser Thr Asn Asp Trp Met Arg Thr Leu Asp Lys Ser Gly Cys Lys Pro
        35                  40                  45
```

```
Arg Asp Thr Val Val Tyr Leu Gly Glu Glu Tyr Pro Glu Ser Thr Asn
    50                  55                  60

Leu Gln Tyr Asn Pro Arg Cys Val Thr Val Lys Arg Cys Ser Gly Cys
65                  70                  75                  80

Cys Asn Gly Asp Gly Gln Ile Cys Thr Ala Val Glu Thr Arg Asn Thr
                85                  90                  95

Thr Val Thr Val Ser Val Thr Gly Val Ser Ser Ser Ser Gly Thr Asn
            100                 105                 110

Ser Gly Val Ser Thr Asn Leu Gln Arg Ile Ser Val Thr Glu His Thr
        115                 120                 125

Lys Cys Asp Cys Ile Gly Arg Thr Thr Thr Thr Pro Thr Thr Thr Arg
    130                 135                 140

Glu Pro Arg Arg
145
```

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala
            20                  25
```

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GCAGAGCTCG TTTAGTGAAC 20

Claims

1. A truncated VRP subunit having a deletion of at least one of the amino acid residues N-terminal to the first cysteine of the core sequence of said subunit.

2. The truncated VRP subunit of claim 1 wherein the VRP is a human VRP.

3. The truncated VRP subunit of claim 1 wherein said VRP is selected from the group consisting of VEGF-B, VRF-2, VEGF-C, PlGF, VEGF-3, poxvirus ORF-1, and poxvirus ORF-2.

4. The truncated VRP subunit of claim 1 wherein said VRP is VEGF-B.

5. The truncated VRP subunit of claim 1 wherein said VRP subunit comprises an amino acid sequence of Figure 2.

6. The truncated VRP subunit of claim 1 wherein the amino acid residues N-terminal to the first cysteine of the core sequence of said subunit are deleted.

7. The truncated VRP subunit of claim 1 wherein the amino acid sequence N-terminal to said core sequence comprises 2 to 5 amino acid residues.

8. The truncated VRP subunit of claim 7 wherein said 2 to 5 amino acid residues comprise 2 to 5 of the consecutive amino acid residues immediately N-terminal to the first cysteine of the core sequence of said VRP subunit.

9. The truncated VRP subunit of claim 1 wherein the amino acid sequence N-terminal to said core sequence comprises 6 to 10 amino acid residues.

10. The truncated VRP subunit of claim 1 wherein said 6 to 10 amino acid residues comprise 6 to 10 of the consecutive amino acid residues immediately N-terminal to the first cysteine of the core sequence of said VRP subunit.

11. The truncated VRP subunit of claim 1 wherein the amino acid sequence N-terminal to said core sequence comprises 11 to 20 amino acid residues.

12. The truncated VRP subunit of claim 1 wherein said 11 to 20 amino acid residues comprise 11 to 20 of the consecutive amino acid residues immediately N-terminal to the first cysteine of the core sequence of said VRP subunit.

13. The truncated VRP subunit according to claim 1, further comprising at the N-terminus of said truncated VRP subunit, the first one or two amino acid residues of the mature non-truncated VRP subunit.

14. A truncated VRP comprising two VRP subunits of claim 13.

15. A truncated VRP comprising two VRP subunits of claim 1, wherein said two VRP subunits have the same amino acid sequence.

16. A truncated VRP heterodimer comprising

a first subunit comprising a truncated VRP subunit of claim 1; and

a second subunit comprising a subunit selected from the group consisting of VRP subunits, and a truncated VRP subunit of claim 1, wherein said second subunit has a different amino acid sequence than said first subunit.

17. A nucleic acid molecule coding for a truncated VRP subunit of claim 1.

18. The nucleic acid molecule of claim 17 wherein the nucleic acid molecule is a DNA molecule.

19. The nucleic acid molecule of claim 17 wherein the nucleic acid molecule is an RNA molecule.

20. A recombinant DNA vector comprising the nucleic acid molecule of claim 17.

21. A recombinant DNA expression vector comprising a nucleic acid molecule of claim 17.

22. The recombinant DNA expression vector of claim 21 wherein said nucleic acid molecule is operably linked at the 5' end of said nucleic acid molecule to a DNA sequence that codes for a signal peptide.

23. The recombinant DNA expression vector of claim 22 wherein said signal peptide is selected from the group consisting of VEGF signal peptide, VEGF-B signal peptide, VRF-2 signal peptide, VEGF-C signal peptide, VEGF-3 signal peptide, and PlGF signal peptide.

24. The recombinant DNA expression vector of claim 22 wherein said signal peptide is selected from the group consisting of poxvirus ORF-1 signal peptide, and poxvirus ORF-2 signal peptide.

25. The recombinant DNA expression vector of claim 22 wherein said signal peptide is VEGF-B signal peptide.

26. The recombinant DNA expression vector of claim 22 wherein said DNA sequence coding for said signal peptide is operably linked at the 3' end of said DNA sequence to DNA coding for the first amino acid residue of the mature non-truncated VRP subunit and wherein the 3' end of said DNA coding for said residue is operably linked to said nucleic acid molecule coding for said truncated VRP subunit.

27. The recombinant DNA expression vector of claim 22 wherein said DNA sequence coding for said signal peptide is operably linked at the 3' end of said DNA sequence to DNA coding for the first two amino acid residues of the mature non-truncated VRP subunits and wherein the 3' end of said DNA coding for said two residues is operably linked to said nucleic acid molecule coding for said truncated VRP subunit.

28. The recombinant DNA expression vector of claim 22 wherein said nucleic acid molecule is operably linked to control sequences operable in a host cell transformed with said vector.

29. A transformed or transfected host cell comprising the recombinant DNA expression vector of claim 21.

30. A transformed or transfected host cell comprising the recombinant DNA expression vector of claim 22.

31. A transformed or transfected host cell comprising the recombinant DNA expression vector of claim 26.

32. A delivery vector comprising a nucleic acid molecule of claim 17.

33. A delivery vector of claim 32, wherein said delivery vector is a viral delivery vector.

34. An adenovirus vector comprising the nucleic acid molecule of claim 17.

35. The adenovirus vector of claim 34 wherein said nucleic acid molecule is operably linked at the 5' end of said nucleic acid molecule to a DNA sequence that codes for a signal peptide.

36. The adenovirus vector of claim 35 wherein said signal peptide is selected from the group consisting of VEGF signal peptide, VEGF-B signal peptide, VRF-2 signal peptide, VEGF-C signal peptide, and PlGF signal peptide.

37. The adenovirus vector of claim 35 wherein said signal peptide is selected from the group consisting of poxvirus ORF-1 signal peptide, and poxvirus ORF-2 signal peptide.

38. The adenovirus vector of claim 35 wherein said signal peptide is VEGF-B signal peptide.

39. The adenovirus vector of claim 35 wherein said DNA sequence coding for said signal peptide is operably linked at the 3' end of said DNA sequence to DNA coding for the first amino acid residue of the mature non-truncated VRP subunit, and wherein the 3' end of said DNA coding for said residue is operably linked to said nucleic acid molecule coding for said truncated VRP subunit.

40. A filtered injectable adenovirus vector preparation, comprising: a recombinant adenoviral vector, said vector containing no wild-type virus and comprising:

a partial adenoviral sequence from which the E1A/E1B genes have been deleted, and

a transgene coding for a truncated VRP subunit of claim 1, driven by a promoter flanked by the partial adenoviral sequence; and

a pharmaceutically acceptable carrier.

41. The preparation of claim 40 wherein said adenovirus vector has been filtered through a 30 micron filter.

42. The injectable adenoviral vector preparation according to claim 40 wherein said promoter is selected from the group consisting of a CMV promoter, a ventricular myocyte-specific promoter, and a myosin heavy chain promoter.

43. A method of producing a truncated VRP polypeptide comprising growing, under suitable conditions, a host cell transformed or transfected with the recombinant DNA expression vector of claim 21 in a manner allowing expression of said polypeptide, and isolating said polypeptide from the host cell.

44. A pharmaceutical composition comprising a VRP comprising at least one truncated VRP subunit of claim 1, in a suitable carrier.

45. A method of stimulating blood vessel formation comprising administering to a patient a pharmaceutical composition comprising a truncated VRP comprising at least one truncated VRP subunit of claim 1, in a suitable carrier.

46. A method of stimulating endothelial cell growth or cell migration in vitro comprising treating said endothelial cells with a truncated VRP comprising at least one truncated VRP subunit of claim 1, in a suitable carrier.

47. A method of treating a patient suffering from a heart disease comprising administering to said patient a nucleic acid molecule coding for at least one truncated VRP subunit of claim 1, said nucleic acid molecule capable of expressing the truncated VRP subunit in said patient.

48. A method of stimulating angiogenesis in a patient comprising administering a therapeutically effective amount of a pharmaceutical composition comprising a truncated VRP comprising at least one truncated VRP subunit of claim 1, in a suitable carrier.

49. The method of claim 48 further comprising a therapeutically suitable delivery system for said pharmaceutical composition.

50. The method of claim 48 further comprising administering a potentiating agent that potentiates the angiogenic effect of said truncated VRP.

51. The method of claim 50, wherein said potentiating agent is an angiogenic FGF.

52. The method of claim 51, wherein said potentiating agent is selected from the group consisting of FGF-1, FGF-2, FGF-4, FGF-5, and FGF-6.

53. A pharmaceutical composition comprising a truncated VRP comprising at least one truncated VRP subunit of claim 1,

and one or more potentiating agents in a pharmaceutically acceptable carrier.

54. The pharmaceutical composition of claim 53 wherein said potentiating agent is an angiogenic FGF.

55. The pharmaceutical composition of claim 54, wherein said potentiating agent is selected from the group consisting of FGF-1, FGF-2, FGF-4, FGF-5, and FGF-6, in a pharmaceutically acceptable carrier.

56. A method of treating a patient suffering from an ischemic condition comprising administering a therapeutic amount of a pharmaceutical composition comprising a truncated VRP comprising at least one truncated VRP subunit of claim 1, in a suitable carrier.

57. The method of claim 56 further comprising administering an agent that potentiates the therapeutic effect of said truncated VRP subunit.

58. The method of claim 57 wherein said potentiating agent is selected from the group consisting of FGF-1, FGF-2, FGF-4, FGF-5, and FGF-6.

59. The method of claim 56 wherein said ischemic condition is selected from the group consisting of: cardiac infarction, chronic coronary ischemia, chronic lower limb ischemia, stroke, and peripheral vascular disease.

60. A method for treating a patient suffering from a wound comprising administering a therapeutic amount of a pharmaceutical composition comprising a truncated VRP

comprising at least one truncated VRP subunit according to claim 1, in a suitable carrier.

61. A method of increasing vascular permeability comprising administering a therapeutic amount of a pharmaceutical composition comprising a truncated VRP comprising at least one truncated VRP subunit according to claim 1, in a suitable carrier.

62. A method of stimulating angiogenesis in a patient comprising delivering a delivery vector to the myocardium of the patient by intracoronary injection directly into one or both coronary arteries, said vector comprising a nucleic acid molecule coding for at least one truncated VRP subunit according to claim 1, wherein said vector is capable of expressing the truncated VRP subunit in the myocardium.

63. The method of claim 62, wherein said delivery vector is a replication-deficient adenovirus vector.

64. A method for stimulating coronary collateral vessel development in a patient having myocardial ischemia, comprising delivering a delivery vector to the myocardium of the patient by intracoronary injection directly into one or both coronary arteries, said vector comprising a nucleic acid molecule coding for a truncated VRP subunit and capable of expressing the truncated VRP subunit in the myocardium, thereby promoting coronary collateral vessel development.

65. The method of claim 64, wherein said delivery vector is a replication-deficient adenovirus vector.

66. A method for stimulating vessel development in a patient having peripheral vascular disease, comprising

delivering a delivery vector to the peripheral vascular system of the patient by intra-femoral artery injection directly into one or both femoral arteries, said vector comprising a transgene coding for a truncated VRP subunit, and capable of expressing the truncated VRP subunit in the peripheral vascular system, thereby promoting peripheral vascular development.

67. The method of claim 66, wherein said delivery vector is a replication-deficient adenovirus vector.

Figure 1

```
hVEGFC                                                mhllgffsvacsllaaallpGPREAPAAAAAFESGLDLSDAEP

hVEGFB                                           mspllrrlllvallqlartqaPVSQFDGPSHQKKVVPWIDVYTRAT
hVRF2                                            mspllrrlllaallqlapaqaPVSQPDAPGHQRKVVSWIDVYTRAT
hVEGFC     DAGEATAYASKDLEEQLRSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILKSIDNEWRKTQ
hPlGF                                         mpvmrlfpcflqllaglalpAVPPQQWALSAGNGSSEVEVVPFQEVWGRSY
hVEGF3                                           mrrcrisgrppappgvpaqaPVSQPDAPGHQRKVVSWIDVYTRAT
pvORF1                                                 MKLLVGILVAVCLHQYLLNADSNTKGWSEVLKGSE
pvORF2                                         MKLTATLQVVVALLICMYNLPECVSQSNDSPPSTNDWMRTLDKSG

hVEGFB     CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQ---------CEC
hVRF2      CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQ---------CEC
hVEGFC     CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTS------CRC
hPlGF      CRPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNVTMQIMRIKPHQSQHIGEMSFLQHSK--------CEC
hVEGF3     CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQ---------CEC
pvORF1     CKPRPIVVPVSETHPELTSQRFNPPCVTLMRCGGCCNDESLECVPTEEVNVTMELLGASGSGSNGMQRLSFVEHKK--------CDC
pvORF2     CKPRDTVVYLGEEYPESTNLQYNPRCVTVKRCSGCCNGDGQICTAVETRNTTVTVSVTGVSSSSGTNSGVSTNLQRISVTEHTKCDC

hVEGFB     RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
hVRF2      RPKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSALTPGPAAAAADAAASSVAKGGA
hVEGFC     MSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAG
hPlGF      RPLREKMKPERRRPKGRGKRRREKQRPTDCHLCGDAVPRR
hVEGF3     RPKKKDSAVKQDRAATPHHRPQPRSVPGWDSAPGAPSPADITQSHSSPRPLCPRCTQHHQCPDPRTCRCRCRRRSFLRCQGRGLELN
pvORF1     RPRFTTTPPTTTRPPRRRR
pvORF2     IGRTTTTPTTTREPRR

hVEGFC     LRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYR
hVEGF3     PDTCRCRKLRR

hVEGFC     RPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS
```

Figure 2a
VEGF-B

F/L	PVSQFDGPSHQKKVVPWIDVYTRAT
(1)	PSHQKKVVPWIDVYTRAT
(2)	KVVPWIDVYTRAT
(3)	PWIDVYTRAT
(4)	IDVYTRAT
(5)	YTRAT
(6)	RAT
F/L	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(1)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(2)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(3)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(4)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(5)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
(6)	CQPREVVVPLSMELMGNVVKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIQYPSSQLGEMSLEEHSQCEC
F/L	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(1)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(2)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(3)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(4)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(5)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK
(6)	RPKKKESAVKPDSPRILCPPCTQRRQRPDPRTCRCRCRRRRFLHCQGRGLELNPDTCRCRKPRK

Figure 2b
VRF-2

```
F/L   PVSQPDAPGHQRKVVSWIDVYTRAT
(1)            PGHQRKVVSWIDVYTRAT
(2)                 KVVSWIDVYTRAT
(3)                      IDVYTRAT
(4)                           RAT

F/L   CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(1)   CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(2)   CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(3)   CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(4)   CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC

F/L   RPKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSALTPGPAAAAADAAASSVAKGGA
(1)   RPKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSALTPGPAAAAADAAASSVAKGGA
(2)   RPKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSALTPGPAAAAADAAASSVAKGGA
(3)   RPKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSALTPGPAAAAADAAASSVAKGGA
(4)   RPKKDSAVKPDRAATPHHRPQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTSALTPGPAAAAADAAASSVAKGGA
```

Figure 2c
VEGF-3

F/L	PVSQPDAPGHQRKVVSWIDVYTRAT
(1)	PGHQRKVVSWIDVYTRAT
(2)	KVVSWIDVYTRAT
(3)	IDVYTRAT
(4)	RAT
F/L	CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(1)	CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(2)	CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(3)	CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
(4)	CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQHQVRMQILMIRYPSSQLGEMSLEEHSQCEC
F/L	RPKKKDSAVKQDRAATPHHRPQPRSVPGWDSAPGAPSPADITQSHSSPRPLCPRCTQHHQCPDPRTCRCRCRRRSFLRCQGRGLELN
(1)	RPKKKDSAVKQDRAATPHHRPQPRSVPGWDSAPGAPSPADITQSHSSPRPLCPRCTQHHQCPDPRTCRCRCRRRSFLRCQGRGLELN
(2)	RPKKKDSAVKQDRAATPHHRPQPRSVPGWDSAPGAPSPADITQSHSSPRPLCPRCTQHHQCPDPRTCRCRCRRRSFLRCQGRGLELN
(3)	RPKKKDSAVKQDRAATPHHRPQPRSVPGWDSAPGAPSPADITQSHSSPRPLCPRCTQHHQCPDPRTCRCRCRRRSFLRCQGRGLELN
(4)	RPKKKDSAVKQDRAATPHHRPQPRSVPGWDSAPGAPSPADITQSHSSPRPLCPRCTQHHQCPDPRTCRCRCRRRSFLRCQGRGLELN
F/L	PDTCRCRKLRR
(1)	PDTCRCRKLRR
(2)	PDTCRCRKLRR
(3)	PDTCRCRKLRR
(4)	PDTCRCRKLRR

Figure 2d

VEGF-C

```
F/L                                                                                            GPREAPAAAAAFESGLDLSDAEP

F/L   DAGEATAYASKDLEEQLRSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILKSIDNEWRKTQ
(1)                                                                                          HYNTEILKSIDNEWRKTQ
(2)                                                                                               ILKSIDNEWRKTQ
(3)                                                                                                    DNEWRKTQ
(4)                                                                                                         KTQ
                                                                                            113   118   123  128

F/L   CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRC
(1)   CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRC
(2)   CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRC
(3)   CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRC
(4)   CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRC

F/L   MSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAG
(1)   MSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAG
(2)   MSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAG
(3)   MSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAG
(4)   MSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAG

F/L   LRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYR
(1)   LRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYR
(2)   LRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYR
(3)   LRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYR
(4)   LRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYR

F/L   RPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS
(1)   RPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS
(2)   RPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS
(3)   RPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS
(4)   RPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS
```

Figure 2e
pVORF1

```
F/L                                         MKLLVGILVAVCLHQYLLNADSNTKGWSEVLKGSE
(1)                                                   LNADSNTKGWSEVLKGSE
(2)                                                      NTKGWSEVLKGSE
(3)                                                           SEVLKGSE
(4)                                                                GSE

F/L   CKPRPIVVPVSETHPELTSQRFNPPCVTLMRCGGCCNDESLECVPTEEVNVTMELLGASGSGSNGMQRLSFVEHKKCDC
(1)   CKPRPIVVPVSETHPELTSQRFNPPCVTLMRCGGCCNDESLECVPTEEVNVTMELLGASGSGSNGMQRLSFVEHKKCDC
(2)   CKPRPIVVPVSETHPELTSQRFNPPCVTLMRCGGCCNDESLECVPTEEVNVTMELLGASGSGSNGMQRLSFVEHKKCDC
(3)   CKPRPIVVPVSETHPELTSQRFNPPCVTLMRCGGCCNDESLECVPTEEVNVTMELLGASGSGSNGMQRLSFVEHKKCDC
(4)   CKPRPIVVPVSETHPELTSQRFNPPCVTLMRCGGCCNDESLECVPTEEVNVTMELLGASGSGSNGMQRLSFVEHKKCDC

F/L   RPRFTTTPPTTTRPPRRRR
(1)   RPRFTTTPPTTTRPPRRRR
(2)   RPRFTTTPPTTTRPPRRRR
(3)   RPRFTTTPPTTTRPPRRRR
(4)   RPRFTTTPPTTTRPPRRRR
```

Figure 2f
pVORF2

```
F/L                                                 MKLTATLQVVVALLICMYNLPECVSQSNDSPPSTNDWMRTLDKSG
(1)                                                                          NDSPPSTNDWMRTLDKSG
(2)                                                                                STNDWMRTLDKSG
(3)                                                                                     MRTLDKSG
(4)                                                                                          KSG

F/L    CKPRDTVVYLGEEYPESTNLQYNPRCVTVKRCSGCCNGDGQICTAVETRNTTVTVSVTGVSSSSGTNSGVSTNLQRISVTEHTKCDC
(1)    CKPRDTVVYLGEEYPESTNLQYNPRCVTVKRCSGCCNGDGQICTAVETRNTTVTVSVTGVSSSSGTNSGVSTNLQRISVTEHTKCDC
(2)    CKPRDTVVYLGEEYPESTNLQYNPRCVTVKRCSGCCNGDGQICTAVETRNTTVTVSVTGVSSSSGTNSGVSTNLQRISVTEHTKCDC
(3)    CKPRDTVVYLGEEYPESTNLQYNPRCVTVKRCSGCCNGDGQICTAVETRNTTVTVSVTGVSSSSGTNSGVSTNLQRISVTEHTKCDC
(4)    CKPRDTVVYLGEEYPESTNLQYNPRCVTVKRCSGCCNGDGQICTAVETRNTTVTVSVTGVSSSSGTNSGVSTNLQRISVTEHTKCDC

F/L    IGRTTTTPTTTREPRR
(1)    IGRTTTTPTTTREPRR
(2)    IGRTTTTPTTTREPRR
(3)    IGRTTTTPTTTREPRR
(4)    IGRTTTTPTTTREPRR
```